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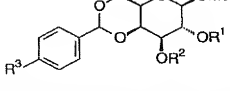
(54) Title: METHODS FOR SYNTHESIS OF α-D-GAL (1→3) GAL-CONTAINING OLIGOSACCHARIDES



(II)

(57) Abstract: This invention relates to reagents and methods for synthesis of biologically active di- and tri-saccharides comprising α-D-Gal(1→3)-D-Gal. In particular the invention provides novel reagents, intermediates and processes for the solution or solid phase synthesis of α-D-galactopyranosyl-(1→3)-D-galactose, and derivatives thereof. In one preferred embodiments the invention provides a protected monosaccharide building block of general formula (II): in which R^3 is methoxy or methyl; R^1 is H, benzoyl, pivaloyl, , 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methylbenzoyl, benzyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-chlorobenzyl, 4-acetamidobenzyl, or 4-azidobenzyl, and R^2 is H, Fmoc, benzoyl, pivaloyl, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methylbenzoyl, benzyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-chlorobenzyl, 4-acetamidobenzyl, or 4-azidobenzyl.

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METHODS FOR SYNTHESIS OF α-D-GAL(1→3) GAL-CONTAINING OLIGOSACCHARIDES

FIELD OF THE INVENTION

This invention relates to methods for synthesis of biologically active di- and tri-saccharides comprising α-D-Gal(1→3)-D-Gal. In particular the invention provides novel reagents, intermediates and processes for the solution or solid phase synthesis of α-D-galactopyranosyl-(1→3)-D-galactose, and derivatives thereof.

BACKGROUND OF THE INVENTION

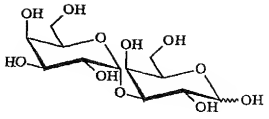
The advent of methods for successful organ transplantation has led to an increasing shortage of donor organs suitable for clinical application. Immuno-concordant species such as non-human primates are potentially a source of allografts which would provide the lowest immunological barrier, but limited availability and ethical concerns, as well as the risk presented by primate retroviruses, mean that this source does not provide a long term solution. Xenografts from discordant but more readily available species, such as pigs, are usually rejected almost immediately. This phenomenon is known as hyperacute rejection (HAR). Thus the suppression of xenoreactive natural antibodies is a key procedure in the implementation of successful xenotransplantation (Tong, Z. *et al*, 1998). It has been reported that ligands comprising the non-reducing terminal oligosaccharides Galα(1→3)Gal and Galα(1→3)Galβ(1→4)GlcNAc showed the highest affinity with human anti-porcine antibodies (Good, H. *et al*. 1992). Of the various means proposed for overcoming HAR, the simplest in concept are the competitive blocking of Galα(1→3)Gal antibodies *in vivo*, or the extracorporeal removal of these antibodies from the circulation (Simon, P.M., 1996). Both methods require the ready availability of the disaccharide or trisaccharide.

In addition to this problem, intestinal infection by *Clostridium difficile* is one of the most common causes of diarrhoea in hospital patients, especially in the elderly (Boriello, S.P., 1990). *C. difficile* has been found to be an aetiological agent of antibiotic-associated diarrhoea and pseudomembranous colitis (Smith, J.A. *et al.*, 1997). *C. difficile* produces two toxins, toxin A and toxin B. Of these, toxin A was shown in animal studies to be an enterotoxin that elicits increased intestinal permeability, fluid secretion and inflammation, and causes severe disruption of the intestinal epithelium (Burakoff, R. *et al*, 1995; Castex, F. *et al*, 1994; Eglow, R. *et al.*, 1992; Torres, J. *et al*, 1990). In model animal systems, the carbohydrate moiety to which toxin A binds has been shown to terminate in the trisaccharide sequence Galα(1$\rightarrow$3)Galβ(1$\rightarrow$4)GlcNAc (Krivan, H.C. *et al*, 1986).

Although the chemistry and biochemistry of oligosaccharide compounds has been extensively studied, there are still difficulties associated with their synthesis and purification. Consequently there is a need in the art for improved methods of synthesis and purification of these compounds.

Apart from the design of effective building blocks, one of the most difficult steps in the synthesis of Galα(1$\rightarrow$3)Gal, Galα(1$\rightarrow$3)Galβ(1$\rightarrow$4)GlcNAc and related compounds is the formation of the α(1$\rightarrow$3) linkage. Although a number of synthetic routes have been described, all of these methods are complex, time-consuming, and costly, and are unsuited to large-scale synthesis.

Chacon-Fuertes provided a procedure for the synthesis of 3-*O*-α-D-galactopyranosyl-D-galactose [i]



(i)

which required a mercuric cyanide-catalysed glycosylation for formation of the α(1→3) glycosidic linkage (Chacon-Fuertes M.E. and Martin-Lomas, M., 1975). The synthesis was protracted, required chromatography, and used dangerous reagents.

Lemieux described the chemical synthesis of 3-*O*-α-D-galactopyranosyl-D-galactose using a *per*-*O*-benzylated α-D-galactopyranosyl bromide sugar donor and a 2,2,2-trichloroethyl 2,4,6-tri-*O*-acetyl-β-D-galact-opyranoside acceptor (Lemieux, R.U. and Driguez, H., 1975). Lemieux employed tetraethyl ammonium bromide as a promoter in a reaction that after chromatography gave 35% yield of product. ^{1}H NMR spectroscopy indicated that the glycosylation product still contained substantial impurities. After deprotection with zinc/acetic acid and preparative thin layer chromatography, de-*O*-acetylation, hydrogenolysis and paper chromatography, an authentic sample of 3-*O*-α-D-galactopyranosyl-D-galactose was finally achieved.

An alternative approach used an allyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside acceptor and an acetimidate sugar donor (Sinaÿ, P. and Jacquinet, J.C., 1979). The formation of the α(1→3) linkage was effected with toluene sulphonic acid in nitromethane in good yield, but chromatography was required for purification. Although generally maintaining yields of greater than 90% for the remainder of the synthesis to the target 3-*O*-α-D-galactopyranosyl-D-galactose, chromatography was required

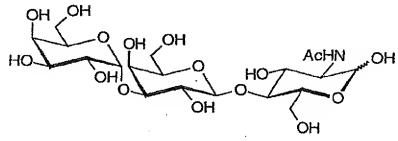
at most steps. Similarly a benzylated Gal(α1-3)Gal disaccharide was synthesised using an α-D-galactopyranosyl bromide donor, but employing stannylene chemistry to selectively activate the 3-*O*-position of the acceptor galactoside, (Augé, C. and Veyrières, A., J.C.S., 1979). The benzylated Galα(1→3)Gal disaccharide subsequently underwent hydrogenolysis to afford 3-*O*-α-D-galactopyranosyl-D-galactose. The reported yields were very low, and most steps required chromatography.

Another synthesis of the 3-*O*-α-D-galactosyl-D-galactose disaccharide employed a benzyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside acceptor and a fully-benzylated imidate galactosyl donor (Milat, M-L. *et al*, 1982). The free disaccharide was eventually obtained after a final hydrogenolysis, and although reasonable yields were achieved, chromatography was unavoidable at many stages of the synthesis. Takeo employed a galactosyl bromide donor and tetraethylammonium bromide as a promoter, and synthesised the disaccharide of interest in a protected form in 40% yield after chromatography. Hydrogenolysis then yielded 3-*O*-α-D-galactopyranosyl-D-galactose (Takeo, K. and Maeda, H., 1988). A chemo-enzymatic synthesis utilised α-D-galactosidase from coffee beans to form the disaccharide, in unreported yield. *p*-Nitrophenyl-α-D-galactopyranoside was used as both the acceptor and donor. The resultant disaccharide derivative was then modified and chromatographed to afford 3-*O*-α-D-galactopyranosyl-D-galactose (Matsuo, I. *et al*, 1997).

It is desirable to avoid the use of toxic reagents, and in order to reduce costs it is also highly desirable to minimise the number of purification steps. If possible, it is particularly desirable to minimize the number of chromatographic purification steps, or even to avoid entirely the need for chromatographic purification, because this technique is time-consuming and costly.

Synthesis of the trisaccharide α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-*N*-acetyl-D-glucosamine

(ii) has understandably been even more difficult than that of α-D-galactopyranosyl-(1→3)-D-galactose.



(ii)

There have been no methods reported in the literature for the synthesis of (ii) using chemical means, although closely analogous compounds have been developed for *in vitro* and *in vivo* applications (Garegg, P.J. and Oscarson, S., 1985; Schaubach, R. *et al*, 1991). There have been some reports of enzymatic synthesis of oligosaccharide (ii) and derivatives thereof. Nilsson synthesised the 2-*N*-trichloroethoxycarbonyl protected ethyl thioglycoside of (ii) by enzymatic methods, using an α-D-galactosidase to effect the formation of the α(1→3) glycosidic linkage followed by β-D-galactosidase treatment (Nilsson, K.G.I., 1997). Similarly galactosidases have been used for the synthesis of target compound (ii), employing similar methodologies (Matsuo, I. *et al*, 1997). Another ethyl thioglycoside derivative of (ii) was synthesised using α and β galactosidases (Vic, G. *et al*, 1997). Analogues of (ii) similar to those described above with lipophilic tails attached *via* the glycosidic linkage were synthesised using α(1→3) galactosyltransferases (Sujino, K. *et al.*, 1998).

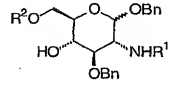
All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly

understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

We have now found that novel thioacyl-substituted glycosides of 3-*O*-α-D-galactopyranosyl-D-galactose can be used for glycoconjugate synthesis by chemical methods. These derivatives can be linked to a suitable solid support, such as polyethylene glycol. These compounds can be used for removal of anti-Gal antibodies from a transplant recipient's blood prior to xenotransplantation, or as anti-bacterial agents to combat bacteria such as *C. difficile*.

SUMMARY OF THE INVENTION

In a first aspect the invention provides a protected glucosamine compound of general formula I:

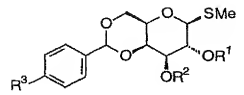


I

in which R^1 is H or acetyl and R^2 is benzyl or 4-chlorobenzoyl,

with the proviso that when R^2 is benzyl, R^1 is not acetyl.

In a second aspect, the invention provides a protected monosaccharide building block of general formula II:



in which R^3 is H, methoxy or methyl, and in which

(a) when R^3 is methoxy or methyl, R^1 is H, benzoyl, pivaloyl, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methylbenzoyl, benzyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-chlorobenzyl, 4-acetamidobenzyl, or 4-azidobenzyl; and

R^2 is H, Fmoc, benzoyl, pivaloyl, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methylbenzoyl, benzyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-chlorobenzyl, 4-acetamidobenzyl, or 4-azidobenzyl;

(b) when R^3 is H, R^1 is benzoyl, pivaloyl, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, benzyl, 3,4-methylene-dioxybenzyl, 4-methoxybenzyl, 4-chlorobenzyl, 4-acetamidobenzyl, or 4-azidobenzyl, and

R^2 is Fmoc, benzoyl, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methylbenzoyl, benzyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-chlorobenzyl, 4-acetamidobenzyl, or 4-azidobenzyl,

with the provisos that

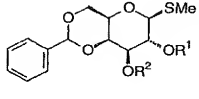
(i) when R^1 is acetyl, R^2 is not chloroacetyl or acetyl, and *vice versa*;

(ii) when R^2 is levulinoyl, R^1 is not benzoyl, and *vice versa*; and

(iii) when R^1 is benzoyl, R^2 is not benzoyl, and *vice versa*.

When R^2 is Fmoc, R^1 is benzoyl, pivaloyl, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methylbenzoyl, benzyl, 3,4-methylene-dioxybenzyl, 4-methoxybenzyl, 4-chlorobenzyl, 4-acetamidobenzyl, or 4-azidobenzyl.

Preferably the compound is of general formula III:



III

in which R^1 is pivaloyl, benzoyl, 4-chlorobenzoyl, 4-methoxybenzyl, or 3,4-methylenedioxybenzyl, and

R^2 is H, Fmoc, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methoxybenzyl, or 3,4-methylenedioxybenzyl, with the proviso that if R^1 is benzoyl, R^2 is not levulinoyl.

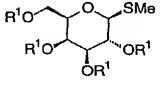
In preferred embodiments, the compound is

(a) a galactopyranoside of general formula III, in which R^1 is 4-chlorobenzoyl, pivaloyl or acetyl, and R^2 is Fmoc or H;

(b) a compound of general formula III in which R^1 is 4-chlorobenzoyl and R^2 is chloroacetyl; or

(c) a compound of general formula III in which both R^1 and R^2 are 3,4-methylenedioxybenzyl.

In a third aspect, the invention provides a galactopyranoside compound of general formula IV:

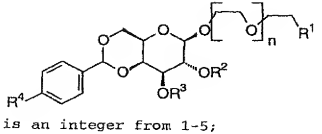


IV

in which each R^1 is independently 4-chlorobenzyl, 4-azidobenzyl, 4-*N*-acetamidobenzyl, 4-methylbenzyl, 3,4-methylenedimethoxybenzyl, or 2-nitrobenzyl.

Preferably each R^1 is 4-chlorobenzyl.

In a fourth aspect the invention provides a polyethyleneglycol (PEG)-linked monosaccharide of general formula V:



V

in which n is an integer from 1-5;

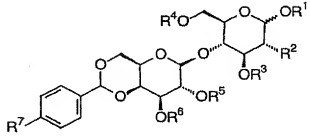
R^1 is a linking group or a group suitable for the formation of a covalent linkage, and includes but is not limited to groups such as halogen, azido, carboxylic acid, thiol, hydroxyl, thioester, xanthate, amido, or dithiocarbamate; R^2 is acetyl, 4-chlorobenzoyl, levulinoyl, pivaloyl, chloroacetate, benzoyl, or 4-methybenzoyl;

R^3 is H, Fmoc, benzoyl, pivaloyl, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methylbenzoyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, or 4-azidobenzyl; and

R^4 is methoxy, H, or methyl.

Preferably n is 2, R^1 is thiobenzoate or thiobiphenylcarbonyl, R^2 is 4-chlorobenzoyl, R^3 is H, and R^4 is H.

In a fifth aspect the invention provides a compound of general formula VI:



VI

in which R^7 is H, methoxy or methyl;

R^1 is aryl, substituted aryl, benzyl, substituted benzyl, alkyl, substituted alkyl, PEG, or substituted PEG;

R^2 is acetamido or amino;

R^3 and R^4 are independently benzyl, substituted benzyl, silylether or acyl;

R^5 is 4-chlorobenzoyl, benzoyl, pivaloyl, acetyl, levulinoyl or 4-methylbenzoyl; and

R^6 is a substituted or unsubstituted pyranosyl or furanosyl sugar, H, Fmoc, acetyl, chloroacetyl, levulinoyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, or 4-azidobenzyl.

When the anomeric configuration of the glucosamine moiety of general formula VI is α and R^3 is benzyl and R^4 is benzoyl and R^7 is H, then R^2 may be acetamido, amino, *N*-phthalimido, R^5 may be 4-chlorobenzoyl, benzoyl, pivaloyl, acetyl, levulinoyl or 4-methylbenzoyl, and R^6 is a substituted or unsubstituted pyranosyl or furanosyl sugar, H, Fmoc, acetyl, chloroacetyl, levulinoyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, or 4-azidobenzyl.

When the anomeric configuration of the glucosamine moiety of general formula VI is β and R^1 is benzyl and R^7 is H, then R^2 is acetamido, amino, or *N*-phthalimido; R^3 and R^4 are independently benzyl, substituted benzyl, silylether or acyl; R^5 is 4-chlorobenzoyl, benzoyl, pivaloyl, acetyl, levulinoyl or 4-methylbenzoyl, and R^6 is a substituted or unsubstituted pyranosyl or furanosyl sugar, H, Fmoc, acetyl, chloroacetyl, levulinoyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, or 4-azidobenzyl.

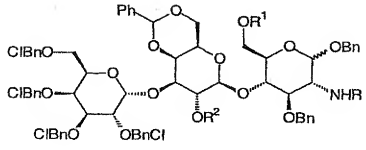
When the anomeric configuration of the glucosamine moiety of general formula VI is α and R^1, R^3, and R^4 are benzyl or substituted benzyl and R^7 is H, then R^2 is acetamido, amino, or *N*-phthalimido, R^5 is pivaloyl, 4-chlorobenzoyl, benzoyl, or levulinoyl, and R^6 is a substituted or unsubstituted pyranosyl or furanosyl sugar, H, Fmoc, acetyl, chloroacetyl, levulinoyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, or 4-azidobenzyl, with the proviso that when R^3 and R^4 are benzyl, R^5 is not acetyl or benzoyl.

In preferred embodiments:

(a) the anomeric configuration of the glucosamine moiety of general formula VI is β, R^1 is benzyl, R^2 is amino or acetamido, R^3 and R^4 are benzyl, R^5 is 4-chlorobenzoyl, pivaloyl or acetyl, R^6 is Fmoc or H, and R^7 is H;

(b) the anomeric configuration of the glucosamine moiety of general formula VI is α, R^1 is benzyl, R^2 is acetamido, R^3 is benzyl, R^4 is benzoyl or benzyl, R^5 is 4-chlorobenzoyl, R^6 is H or 4-chloroacetyl and R^7 is H;

(c) the compound is a trisaccharide of General Formula VII:



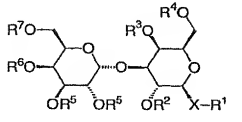
VII

in which R is H or acetyl; R^1 is hydrogen, benzyl, benzoyl or *p*-chlorobenzoyl; and R^2 is hydrogen, 4-chloro-benzoyl, acetyl, benzoyl or pivaloyl;

(d) the compound is a trisaccharide of general formula VII, in which the anomeric configuration of the reducing end is α, R is acetyl, R^1 is benzoyl, 4-chlorobenzoyl or H, and R^2 is 4-chlorobenzoyl or H; or

(e) the compound is a trisaccharide of general formula VII, in which the anomeric configuration of the reducing sugar is β, R is acetyl or H, R^1 is benzyl, and R^2 is H, 4-chlorobenzoyl, pivaloyl or acetyl.

In a sixth aspect the invention provides a compound of general formula VIII:



VIII

in which R^5, R^6 and R^7 are independently H, 4-chlorobenzyl, 4-methoxybenzyl, 4-methylbenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl;

X is O, S, or N;

R^1 is alkyl, substituted alkyl, aryl, substituted aryl, PEG or substituted PEG;

R^2 is levulinoyl, 4-chlorobenzoyl, benzoyl, 4-methylbenzoyl, acetyl or pivaloyl; and

R^3 and R^4 may combine to form a benzylidene ring, which may optionally be substituted at the 4 position by methyl or methoxy; alternatively R^3 and R^4 may independently be H, benzyl or substituted benzyl.

When R^5 is 4-chlorobenzyl, 4-methoxybenzyl, 4-methylbenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl, and R^6 and R^7 combine to form a benzylidene or substituted benzylidene ring, then X is O, S, or N, R^1 is alkyl, substituted alkyl, aryl, substituted aryl, PEG, substituted PEG, acyl or substituted acyl, and R^2 is levulinoyl, 4-chlorobenzoyl, benzoyl, 4-methylbenzoyl, acetyl or pivaloyl.

When X is oxygen and R^1 is 3,4-methylenedioxybenzyl, then R^2 is H, 4-chlorobenzoyl, pivaloyl, acetyl, levulinoyl, benzoyl or chloroacetyl, R^3 and R^4 may combine to become a benzylidene ring or may independently be H, benzyl or substituted benzyl, and R^5, R^6 and R^7 may be H, benzyl, 4-chlorobenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl.

When X is oxygen and R^1 is 2-[2-(2-thiobenzoyl)-ethoxy)ethyl or 2-[2-(2-thiobiphenylcabonyl)ethoxy], then R^2 is H, 4-chlorobenzoyl, pivaloyl, acetyl, levulinoyl, benzoyl or chloroacetyl, R^3 and R^4 may combine to form a benzylidene ring or may independently be H, benzyl, 4-chlorobenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl, R^5 is H, benzyl, 4-chlorobenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl, and R^6 and R^7 may combine to become a benzylidene ring or may independently be H, benzyl, 4-chlorobenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl.

When X is sulphur, R^1 is alkyl, substituted alkyl, aryl or substituted aryl, R^3 and R^4 combine to form a

benzylidene ring and R^5, R^6 and R^7 are benzyl, then R^2 is levulinoyl, 4-chlorobenzoyl, benzoyl, acetyl or pivaloyl, with the proviso that when R^1 is phenyl, R^2 is not levulinoyl.

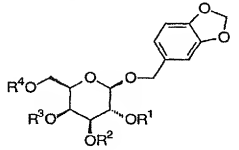
Preferably either

(a) X is oxygen, R^1 is 2-[2-(2-thiobenzoyl)ethoxy)ethyl or 2-[2-(2-thiobiphenylcabonyl)ethoxy], R^2 is H or 4-chlorobenzoyl, R^3 and R^4 are H or combine to form a benzylidene ring, R^5 is H or 3,4-methylenedioxybenzyl, and R^6 and R^7 are both H or combine to form a benzylidene ring;

(b) X is S, R^1 is methyl, R^2 is 4-chlorobenzoyl, R^3 and R^4 combine to form a benzylidene ring, and R^5, R^6 and R^7 are each 4-chlorobenzyl; or

(c) X is oxygen, R^1 is 3,4-methylenedioxybenzyl, R^2 is 4-chlorobenzoyl or H, R^3 and R^4 combine to form a benzylidene ring or are both H, and R^5, R^6 and R^7 are independently 4-chlorobenzyl or H.

In a seventh aspect the invention provides a compound of general formula IX:



IX

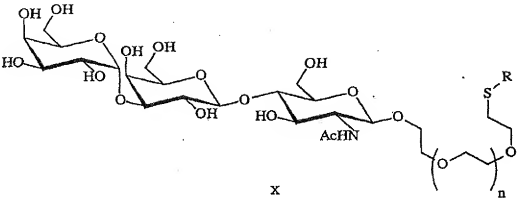
in which R^1 is 4-chlorobenzoyl, pivaloyl, acetyl, levulinoyl, benzoyl or chloroacetyl;

R^2 is H, benzyl, 4-chlorobenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, azidobenzyl, 3,4-methylenedioxybenzyl, Fmoc, levulinoyl, acetyl or chloroacetyl; and

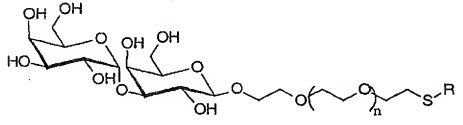
R^3 and R^4 may combine to form a benzylidene ring, or may independently be H, benzyl, 4-chlorobenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl.

Preferably R^1 is 4-chlorobenzoyl, R^2 is H, and R^3 and R^4 combine to form a benzylidene ring.

In an eighth aspect the invention provides a polyethyleneglycol(PEG)-linked disaccharide of General Formula X or a trisaccharide of General Formula XI:



X

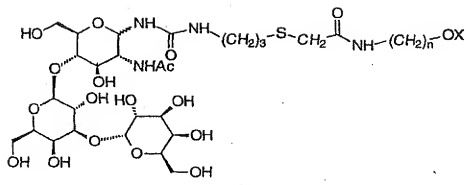


XI

in which R is hydrogen or acyl, and n is an integer of from 1 to 3.

Preferably the compound of General Formula XI is 2-[2-(2-thiobiphenylcarbonyl)ethoxy]-ethyl 3-O-(α-D-galactopyranosyl)-α-galactopyranoside.

In a ninth aspect, the invention provides Galα(1→3)Galβ(1→4)GlcNAc coupled to a solid support to give a compound of general formula XII:



XII

in which X is a solid support such as Sepharose or silica gel, and n is an integer of from 3 to 6.

The compounds of the first seven aspects of the invention are useful as intermediates in the synthesis of di- and trisaccharides. Accordingly, in a tenth aspect, the invention provides a method of synthesis of a desired compound of General Formula X to General Formula XII, or of α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-*N*-acetyl-D-glucosamine (Galα(1→3)Galβ(1→4)GlcNAc), α-D-galactopyranosyl-(1→3)-β-D-galactopyranose (Galα(1→3)Gal), or β-D-galactopyranosyl-(1→4)-*N*-acetyl-D-glucosamine (Galβ(1→4)GlcNAc), comprising the step of using a compound of General Formula I to IX as an intermediate.

Preferably when the desired compound is of general Formula X or XI the intermediate compound is of General Formula V. It will be clearly understood that although a compound of General Formula VI may be synthesised using a compound of General Formula I as an intermediate, alternative syntheses are available.

For the purposes of this specification, the term "alkyl" is intended to include saturated, unsaturated and cyclic hydrocarbon groups, and combinations of such groups.

Suitable substituents on hydrocarbon chains or aryl rings include Br, Cl, F, I, CF_3, NH_2, substituted amino groups such as NHacyl, hydroxy, carboxy, C_{1-6}alkylamino and C_{1-6}alkoxy groups such as methoxy, and are preferably F, Cl, hydroxy, C_{1-6}alkoxy, amino, C_{1-6}alkylamino or C_{1-6}carboxy.

In a eleventh aspect, the invention provides a method of preventing or reducing a hyperacute rejection response associated with xenotransplantation, comprising the step of administering an effective dose of thioalkyl Galα-(1→3)Gal or thioalkyl Galα(1→3)Galβ(1→4)GlcNAc to a subject in need of such treatment.

The compound may be administered before, during or after xenotransplantation.

In a twelfth aspect, the invention provides a method of preventing or reducing hyperacute rejection associated with xenotransplantation, comprising the steps of

a) removing plasma from a patient who is to undergo xenotransplantation;

b) exposing the plasma to thioalkyl Galα(1→3)Gal or thioalkyl Galα(1→3)Galβ(1→4)GlcNAc linked to a solid support, and

c) reinfusing the thus-treated plasma into the patient.

In a thirteenth aspect, the invention provides a method of depleting anti-Galα(1→3)Gal antibodies from a plasma or serum sample, comprising the step of exposing the plasma or serum to thioalkyl Galα(1→3)Gal or thioalkyl Galα(1→3)Galβ(1→4)GlcNAc linked to a solid support.

In a fourteenth aspect, the invention provides a method of treatment of *C. difficile* infection, comprising the step of administering an effective amount of α-D-galactopyranosyl-(1→3)-β-D-galacto-pyranosyl-(1→4)-*N*-acetyl-D-glucosamine (Galα(1→3)Galβ(1→4)GlcNAc) or of thioalkyl Galα(1→3)Galβ(1→4)GlcNAc, preferably linked to a solid support, to a subject in need of such treatment.

Preferably the solid support is a multidentate ligand or a dendrimer compound. Suitable dendrimers are disclosed

for example in International patent application No. PCT/AU95/00350 (WO95/34595) by Biomolecular Research Institute Ltd.

In the eleventh to the fourteenth aspects of the invention, the subject may be a human, or may be a domestic, companion or zoo animal. While it is particularly contemplated that the compounds of the invention are suitable for use in medical treatment of humans, they are also applicable to veterinary treatment, including treatment of companion animals such as dogs and cats, and domestic animals such as horses, cattle and sheep, or zoo animals such as felids, canids, bovids, and ungulates.

Methods and pharmaceutical carriers for preparation of pharmaceutical compositions are well known in the art, as set out in textbooks such as Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, Easton, Pennsylvania, USA.

The compounds and compositions of the invention may be administered by any suitable route, and the person skilled in the art will readily be able to determine the most suitable route and dose for the condition to be treated. Dosage will be at the discretion of the attendant physician or veterinarian, and will depend on the nature and state of the condition to be treated, the age and general state of health of the subject to be treated, the route of administration, and any previous treatment which may have been administered.

The carrier or diluent, and other excipients, will depend on the route of administration, and again the person skilled in the art will readily be able to determine the most suitable formulation for each particular case.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described in detail by way of reference only to the following non-limiting examples.

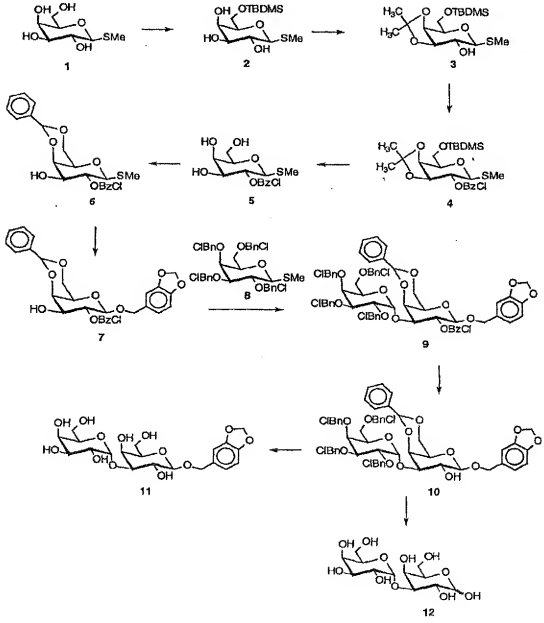
Abbreviations used herein are as follows:

AcN	Acetonitrile
Bn	Benzyl
CH_2Cl_2	Dichloromethane
$CHCl_3$	Chloroform
*p*ClBn	*para*-chlorobenzyl
*p*ClBz	*para*-chlorobenzoyl
DCM	Dichloromethane
DMF	*N,N'*-Dimethylformamide
DMTST	Dimethyl(methylthio)sulphoniumtrifluoromethanesulphonate
EtOAc	Ethyl acetate
EtOH	Ethanol
H_2O	Water
HRMS	High resolution mass spectrometry
MDBn	3,4-methylenedioxybenzyl
Me	Methyl
MeCN	Acetonitrile
MeOH	Methanol
$MgSO_4$	Magnesium sulphate
$NaHCO_3$	Sodium hydrogen carbonate
NMR	Nuclear magnetic resonance
PEG	Polyethylene glycol
Ph	Phenyl
$SOCl_2$	Thionyl chloride
TBDMS	*tertiary*-butyldimethylsilyl
THF	Tetrahydrofuran

Example 1: Preparation of 3,4-Methylenedioxybenzyl 4,6-O-Benzylidene 2-O-(4-chlorobenzoyl)-β-D-Galactopyranoside Acceptor

The strategy for this preparation is set out in Reaction Scheme 1.

Synthesis of α-D-Galactopyranosyl-(1→3)-D-Galactose



Reaction Scheme 1

Methyl 6-O-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside (**2**)

A mixture of t-butyldimethylsilyl chloride (68.35 g, 453.51 mmol) and 4-dimethylaminopyridine (55.40 g, 453.51 mmol) in dry 1,2-dichloroethane (800 ml) was stirred at 80°C for 15 minutes. Methyl 1-thio-β-D-galactopyranoside (**1**) (100 g, 476.19 mmol) was added in 5 portions in 15 minutes to the stirred solution at 80°C, and the reaction mixture was stirred under reflux for 1 hour. The resulting clear solution was cooled to room temperature, diluted with $CHCl_3$ (2 000 ml), washed four times with diluted brine solution (water-brine 2:1) (750 ml). The aqueous layers of the last two washings were collected and extracted with $CHCl_3$ (400 ml). The organic layers were combined, dried over $MgSO_4$ and evaporated. The residue was kept under high vacuum for 15 min, then was dissolved in dry MeCN (200 ml). The solution was evaporated, and the residue was kept under high vacuum for 15 min. This drying process was repeated using another 200 ml of dry MeCN, to give the crude methyl 6-O-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside (**2**) (117.5 g, 80%) as a syrup.

R_f 0.65 (MeCN/H_2O 10:1) MS (electrospray) $C_{13}H_{28}O_5SSi$ (324.23) m/z (%) 347$[M+Na]^+$ (100), 325$[M+H]^+$ (75).

Methyl 6-O-tert-butyldimethylsilyl-3,4-O-isopropylidene-1-thio-β-D-galactopyranoside (**3**)

A mixture of crude methyl 6-O-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside (**2**) (117.46 g, 362.27 mmol), 2,2-dimethoxypropane (66.82 ml, 543.41 mmol) and p-toluenesulphonic acid (200 mg) in dry MeCN (800 ml) was stirred at 40°C for 30 minutes. The reaction mixture was neutralized with triethylamine (3 ml) and evaporated to give a white crystalline residue (**3**) (161.3 g).

R_f 0.62 (EtOAc/Hexane 2:1) MS (electrospray) $C_{16}H_{32}O_5SSi$ (364.58) m/z (%) 387$[M+Na]^+$ (45), 365 $M+H]^+$ (100).

Methyl 6-O-tert-butyldimethylsilyl-2-O-(4-chlorobenzoyl)-3,4-O-isopropylidene-1-thio-β-D-galactopyranoside (4)

A mixture of methyl 6-O-tert-butyldimethylsilyl-3,4-O-isopropylidene-1-thio-β-D-galactopyranoside (3) (155.44 g, 427. 03 mmol) and 4-dimethylaminopyridine (62.60 g, 512.44 mmol) in dry 1,2-dichloroethane (750 ml) was stirred at room temperature. 4-Chlorobenzoyl chloride (89.68 g, 512.44 mmol) was added to the stirred reaction mixture in 15 minutes. After the addition the resulting suspension was stirred under reflux for 30 minutes. The reaction mixture was cooled to 10°C and filtered. The crystalline solid was washed on the funnel with dry 1,2-dichloroethane (300 ml) and filtered. The filtrates were combined, diluted with $CHCl_3$ (2000 ml) and washed twice with diluted brine solution (water-brine 2:1) (1500 ml). The organic layer was dried over $MgSO_4$ and evaporated. The residue was kept under high vacuum for 15 minutes. The resulting syrup was dissolved in dry MeCN (200 ml) and evaporated using high vacuum at the end of the evaporation, to give the crude methyl 6-O-tert-butyldimethylsilyl-2-O-(4-chlorobenzoyl)-3,4-O-isopropylidene-1-thio-β-D-galactopyranoside (4) (165 g) as a colourless syrup.

R_f 0.68 (Hexane/EtOAc 2:1) MS (electrospray) $C_{23}H_{35}O_6SSi$ (503.14), m/z (%) 503$[M+H]^+$ (100), 525$[M+Na]^+$ (38).

Methyl 2-O-(4-chlorobenzoyl)-1-thio-β-D-galactopyranoside (5)

A mixture of methyl 6-O-tert-butyldimethylsilyl-2-O-(4-chlorobenzoyl)-3,4-isopropylidene-β-D-galactopyranoside (4) (173 g, 344.62 mmol) and *p*-toluenesulphonic acid (600 mg) in MeOH-MeCN 3:1 (2000 ml) was stirred under reflux for 1 hour. The reaction mixture was cooled to room temperature and evaporated. The resulting white solid residue was

suspended in diisopropylether (1000 ml) and filtered. The crystalline solid was washed twice with diisopropylether (300 ml), then with diethylether (500 ml) and dried to give methyl 2-O-(4-chlorobenzoyl)-1-thio-β-D-galactopyranoside (5) (107 g) as a white crystalline powder.

R_f 0.45 (MeCN/H_2O 10:1) MS (electrospray) $C_{14}H_{17}ClO_6S$ (348.80) m/z (%) 371$[M+Na]^+$ (35), 349$[M+H]^+$ (100).

Methyl 2-O-(4-chlorobenzoyl)-4,6-O-benzylidene-1-thio-β-D-galactopyranoside (6)

A mixture of methyl 2-O-(4-chlorobenzoyl)-1-thio-β-D-galactopyranoside (5) (94.16 g, 270.57 mmol), α,α-dimethoxytoluene (60.9 ml, 405.86 mmol) and *p*-toluenesulphonic acid (200 mg) in dry MeCN (500 ml) was stirred at 70°C for 30 minutes. The reaction mixture was cooled to room temperature, neutralized with triethylamine (3 ml) and evaporated. The residue was taken up in $CHCl_3$ (1500 ml), washed with diluted brine solution (water- brine 2:1) (750 ml), with saturated $NaHCO_3$ solution (500 ml), with diluted brine again (water-brine 2:1) (750 ml), dried over $MgSO_4$ and evaporated. The resulting white solid was kept under high vacuum for 15 minutes. The dry residue was crystallized from MeCN (250 ml) at room temperature to give 68.5 g pure product. Water (80 ml) was added slowly to the mother liquor, and the solution was left at room temperature to crystallize another 20.8 g of methyl 2-O-(4-chlorobenzoyl)-4,6-O-benzylidene-1-thio-β-D-galactopyranoside (6) (yield: 75%).

R_f 0.65 (EtOAc/Hexane 2:1) MS (electrospray) $C_{21}H_{21}ClO_6S$ (436.91) m/z (%) 437$[M+H]^+$ (56), 459$[M+Na]^+$ (100).

^1H NMR ($CDCl_3$) δ 8.01-7.37 (9H, aromatic), 5.56 (s, 1H, benzylidene), 5.44 (t, 1H, H-2), 4.5 (d, 1H, J_{1-2}=9.0, H-1), 4.38 (dd, 1H, H-6_a), 4.30 (dd, 1H, H-4), 4.04 (dd, 1H, H-

6_b), 3.90 (m, 1H, H-3), 3.6 (s, 1H, H-5), 2.25 (s, 3H, S-CH_3).

3,4-Methylenedioxybenzyl 4,6-O-benzylidene 2-O-(4-chlorobenzoyl)-β-D-galactopyranoside (7)

To a mixture of methyl 4,6-O-benzylidene 2-O-(4-chlorobenzoyl)-1-thio-β-D-galactopyranoside (6) (10 g, 22.9 mmol), 3,4-methylenedioxybenzyl alcohol (5.6 g, 36.8 mmol) and powdered molecular sieves (5Å, 15 g) in dry 1,2-dichloroethane (200 mL) at 0°C, was added methyl trifluoromethanesulphonate (6 g, 36.6 mmol) in one portion under nitrogen atmosphere. The reaction mixture was sealed and left to warm to room temperature, and stirred for 3 h. The mixture was then neutralized with triethylamine (15 mL), diluted with $CHCl_3$ (350 mL) and filtered through celite. The filtrate was washed with saturated $NaHCO_3$ solution (4 x 500 mL), and the organic layer was dried over $MgSO_4$ and evaporated to dryness leaving a white solid. The solid was suspended in diisopropylether (200 mL), filtered, washed with diisopropylether (200 mL) and dried to give 3,4-methylenedioxybenzyl 4,6-O-benzylidene 2-O-(4-chlorobenzoyl)-β-D-galactopyranoside (7) (7.5 g, 61% yield) as a white powder.

R_f 0.60 (CH_2Cl_2/EtOH 20:1) MS (electrospray) $C_{28}H_{25}ClO_9$ (540.95) m/z (%) 437$[M+H]^+$ (56), 558$[M+H+NH_3]^+$ (100).

Example 2: Preparation of Methyl 2,3,4,6-tetra-O-(4-chlorobenzyl)-1-thio-β-D-Galactopyranoside Glycosyl Donor

Methyl 2,3,4,6-tetra-O-(4-chlorobenzyl)-1-thio-β-D-galactopyranoside (8)

To a stirred suspension of sodium hydride (95%) (14.43 g, 571.42 mol) in dry DMF (300 ml) a solution of methyl 1-thio-β-D-galactopyranoside (1) (20 g, 95.23 mmol) in dry DMF (200 ml) was added dropwise at 0°C in nitrogen atmosphere. At the end of the addition the ice-bath was

removed and the reaction mixture was stirred at room temperature for 30 minutes. 4-Chlorobenzyl chloride (97.74 g, 571.42 mmol) was added dropwise to the stirred reaction mixture keeping the temperature 10-20°C. After the addition, the reaction mixture was stirred at room temperature overnight. The resulting suspension was cooled with ice-bath and methanol (11 ml) was added slowly. When the hydrogen formation had stopped, the suspension was evaporated to dryness at 45-50°C. The remaining DMF was removed by co-evaporation with xylene (100 ml). The residue was taken up in CH_2Cl_2 (500 ml), washed twice with water (500 ml), saturated $NaHCO_3$ solution (500 ml), dried over $MgSO_4$ and evaporated. The residue was crystallized from EtOH (500 ml) to give methyl 2,3,4,6-tetra-O-(4-chlorobenzyl)-1-thio-β-D-galactopyranoside (8) (40 g, 60%) as a white crystalline solid.

R_f 0.72 (Hexane/EtOAc 3:1) MS (electrospray) $C_{35}H_{34}Cl_4O_5S$ (708.53) m/z (%) 709[M+H]$^+$ (100), 731[M+Na]$^+$ (48).

Example 3: Preparation of 3-O-(α-D-galactopyranosyl)-D-galactopyranose

3,4-Methylenedioxybenzyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranoside (9)

Methyl trifluoromethanesulphonate (4 g, 24 mmol) was added under nitrogen to a mixture of 3,4-methylenedioxybenzyl 4,6-O-benzylidene 2-O-(4-chlorobenzoyl)-β-D-galactopyranoside (7) (6.5 g, 12 mmol), methyl 2,3,4,6-tetra-O-(4-chlorobenzyl)-thio-β-D-galactopyranoside (8) (12 g, 17 mmol) and powdered molecular sieves (5Å, 10 g) in dry 1,2-dichloroethane (250 mL). The sealed reaction mixture was left to warm to room temperature and then stirred for 80 minutes. The reaction mixture was neutralized with triethylamine (12 g) and diluted with $CHCl_3$ (500 mL). The suspension was filtered through celite and the filtrate was washed with

saturated $NaHCO_3$ solution (3 x 500 mL). The organic phase was dried over $MgSO_4$ and evaporated to dryness to give an oily residue. The residue was suspended in diisopropylether (150 mL) and the resulting solid was filtered. The solid was washed with diisopropylether (100 mL) and dried under high vacuum at room temperature to give 3,4-methylenedioxybenzyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranoside (**9**) (6.7 g, 47%) as a white powder.

R_f 0.50 (EtOAc/Hexane 1:1) MS (electrospray) $C_{62}H_{55}Cl_5O_{14}$ (1201.38) m/z (%)1221[M+Na]$^+$ (80).

3,4-Methylenedioxybenzyl 4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranoside (**10**)

To a solution of sodium methoxide (280 mg, 10.4 mmol) in dry methanol (50 mL), 3,4-methylenedioxy-benzyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranoside (**9**) (6.3 g, 5.2 mmol) in dry THF-MeOH 2:1 (150 mL) was added. The resulting mixture was stirred at 40°C for 5 hours. The reaction mixture was cooled to 18°C and neutralized (pH 7.0) with Amberlite IR-120 H$^+$ cation exchange resin. The resin was filtered off and the filtrate evaporated to dryness to give an oily residue. The crude product was suspended in hexane (200 mL), which was then vigorously stirred to break up the clumps. The suspension was filtered and dried in vacuum at room temperature to give 3,4-methylenedioxybenzyl 4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranoside (**10**) (5.2 g, 93%) as a white powder.

R_f 0.30 (CH_2Cl_2/ethanol 50:1), MS (electrospray) m/z $C_{55}H_{52}Cl_4O_{13}$ (1062.83) m/z (%) 1098[M+K]$^+$ (72)

3,4-methylenedioxybenzyl 3-O-(α-D-galactopyranosyl)-β-D-galactopyranoside (**11**)

To a suspension of Pd/C (10%) catalyst (220 mg) in a mixture of THF-EtOH-H_2O 6:2:1 (5 mL), a solution of 3,4-methylenedioxybenzyl 4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranoside (**10**) (200 mg, 0.19 mmol) in a mixture of THF-EtOH-H_2O 6:2:1 (5 mL) was added. The resulting suspension was shaken under a positive pressure (45 PSI) of hydrogen for 2.5 h. The reaction mixture was filtered through celite and the filtrate was concentrated under high vacuum at room temperature to a volume of approximately 15 mL. The resulting yellow solution was diluted with deionised water (50 mL) and neutralized (pH 7.0) with excess mixed bed resin (Amberlite-MB 1). The aqueous suspension was filtered and the filtrate was evaporated to dryness under high vacuum to give the crude product as a colourless residue. The crude product was purified by chromatography using $CHCl_3$-MeOH-H_2O 5:5:1 as the mobile phase to give 3,4-methylenedioxybenzyl 3-O-(α-D-galactopyranosyl)-β-D-galactopyranoside (**11**) (72 mg, 73%).

R_f 0.42 ($CHCl_3$/MeOH/H_2O 5:5:1) MS (electrospray) $C_{20}H_{28}O_{13}$ (476.43) m/z (%) 499$[M+Na]^+$ (38), 477$[M+H]^+$ (72)

3-O-(α-D-Galactopyranosyl)-D-galactopyranose (**12**)

A mixture of $Pd(OH)_2$ (20%) Pearlman's catalyst (0.7 g) and 3,4-methylenedioxybenzyl 4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranoside (**10**) (2.0 g, 1.9 mmol) in a mixture of THF-MeOH-H_2O 4:1:1 (30 mL) was shaken under a positive pressure (60 PSI) of hydrogen overnight. The reaction mixture was filtered through celite and the filtrate was neutralized with mixed-bed ion exchange resin (Amberlite-MB 1) /negative silver (I) nitrate test/. The reaction mixture

was filtered and the filtrate was concentrated to dryness in *vacuum* at room temperature. The residue was taken up in deionised water (2 mL) and passed through a C18 Sep Pak cartridge eluting with milli-Q-water (30 mL). The filtrate was evaporated under reduced pressure to give 3-O-(α-D-galactopyranosyl)-D-galactopyranose (**12**) (560 mg, 86%) as a white solid foam..

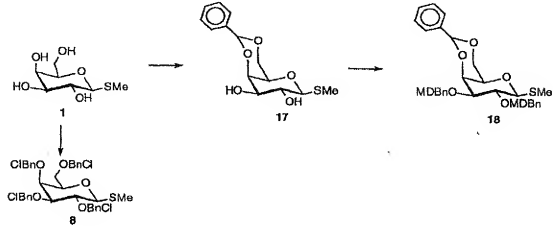
TLC ($CHCl_3$-MeOH-H_2O 10 : 10 : 2) R_f =0.3, High performance anion exhange chromatography with pulsed amperometric detection /HPAE-PAD/ (4 x 250 mm Dionex CarbopaK-PA1 analytical column with guard column, 150 mM sodium hydroxide at 1 mL/min.) t_R =5.0 min., MS (electrospray) m/z 365 $[M + Na]^+$.

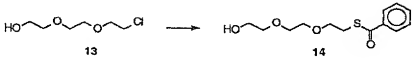
R_f 0.30 ($CHCl_3$/MeOH/H_2O 5:5:1) MS (electrospray) $C_{12}H_{22}O_{11}$ (342.29) m/z (%) 406$[M+Na+MeCN]^+$ (100), 365$[M+Na]^+$ (62)

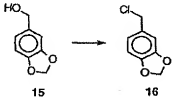
Example 4: Preparation of 2-[2-(2-thiobiphenylcarbonyl)-ethoxy]ethyl 3-O-α-D-galactopyranosyl-β-D-galactopyranoside (23)

The synthesis of the reagents for this preparation and the preparation scheme itself are set out in Reaction Schemes 2 and 3 respectively.

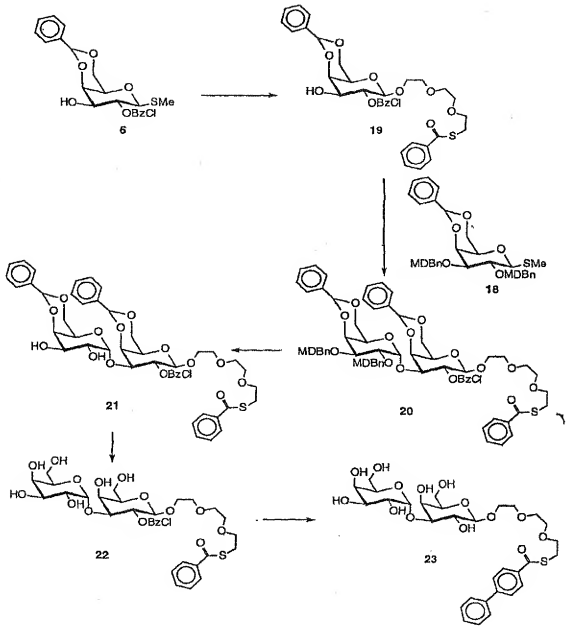
Reagents for the Synthesis of 2-[2-(2-Thiobiphenyl-carbonyl)-ethoxy]ethyl 3-O-α-D-Galactopyranosyl-β-D-Galactopyranoside







Reaction Scheme 2



Reaction Scheme 3

2-[2-(2-Thiobenzoyl)ethoxy]ethanol (**14**)

A mixture of 2-[2-(2-chloroethoxy)ethoxy]ethanol (**13**) (17.1 g, 101 mmol) and cesium thiobenzoate (38.24 g, 142 mmol) in dry DMF (200 ml) was stirred at 75°C for 1.5 hours. The reaction mixture was cooled to room

temperature and evaporated to dryness. The residue was taken up in diethylether (600 ml), washed three times with saturated $NaHCO_3$ solution (400 ml) and with water (500 ml). The organic phase was dried over $MgSO_4$ and evaporated to dryness to give 23 g of crude product. The crude residue was purified by chromatography using diethylether as the mobile phase to give 2-[2-(2-thiobenzoyl)ethoxy]ethanol (14) (18.75 g, 68%) as an orange syrup.

R_f 0.60 (diethylether/EtOH 19:1) MS (electrospray) $C_{13}H_{18}O_4S$ (270.34) m/z (%) 293[M+Na]$^+$ (62), 271[M+H]$^+$ (100)

3,4-Methylenedioxybenzyl chloride (16)

A solution of 3,4-methylenedioxybenzyl alcohol (15) (50 g, 328.62 mmol) in CH_2Cl_2 (50 ml) was cooled to 0°C and $SOCl_2$ (250 ml) added dropwise. The reaction mixture was stirred at 0°C for 1 hour, at room temperature for 4 hours, then evaporated to dryness. The residue was purified by distillation under vacuum to give 3,4-methylenedioxybenzyl chloride (16) (49 g, 87%).

R_f 0.75 ($CHCl_3$/EtOAc 20:1)

Methyl 4,6-O-benzylidene-1-thio-β-D-galactopyranoside (17)

A mixture of methyl 1-thio-β-D-galactopyranoside (1) (23.6 g, 112 mmol), α,α-dimethoxytoluene (25.62 g, 168 mmol) and *p*-toluenesulphonic acid (100 mg) in MeCN (500 ml) was stirred at room temperature for 30 minutes. The reaction mixture was neutralized with triethylamine (1 ml) and evaporated to dryness, followed by a co-evaporation with toluene. The residue was taken up in CH_2Cl_2 (250 ml), washed twice with brine (250 ml), dried over $MgSO_4$ and evaporated. The resulting white solid was crystallized from EtOH to give methyl 4,6-O-benzylidene-1-thio-β-D-galactopyranoside (17) (27.5 g, 82%).

R_f 0.32 (EtOAc) MS (electrospray) $C_{14}H_{18}O_5S$ (298.36) m/z (%) 321[M+Na]$^+$ (32), 299[M+H]$^+$ (100)

Methyl 4,6-O-benzylidene-2,3-di-O-(3,4-methylenedioxybenzyl)-1-thio-β-D-galactopyranoside (**18**)

A mixture of methyl 4,6-O-benzylidene-1-thio-β-D-galactopyranoside (**17**) (20 g, 66.80 mmol) and sodium hydride (95%) (4.80 g, 201.2 mmol) in dry DMF (350 ml) was stirred at 0°C for 30 minutes, then 3,4-methylenedioxybenzyl chloride (34.3 g, 201,2 mmol) (**16**) added in DMF (20 ml). The reaction mixture was stirred at room temperature overnight. Methanol (20 ml) was added and the reaction mixture was evaporated to dryness. The residue was taken up in CH_2Cl_2 (500 ml), washed twice with brine (500 ml), dried over $MgSO_4$ and evaporated. The residue was crystallized from 2-propanol (1 l) to give methyl 4,6-O-benzylidene-2,3-di-O-(3,4-methylenedioxybenzyl)-1-thio-β-D-galactopyranoside (**18**) (19 g, 50%).

R_f 0.62 ($CHCl_3$/EtOAc 20:1), MS (electrospray) $C_{30}H_{30}O_9S$ (566.62) m/z (%) 589[M+Na]$^+$ (100), 567[M+H]$^+$ (25)

2-[2-(2-Thiobenzoyl)ethoxy]ethyl 4,6-O-benzylidene 2-O-(4-chlorobenzoyl)-β-D-galactopyranoside (**19**)

A mixture of methyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-1-thio-β-D-galactopyranoside (**6**) (10 g, 22.93 mmol), 2-[2-(2-thiobenzoyl)ethoxy]ethanol (**13**) (6.81 g, 25.22 mmol), powdered molecular sieves 4Å (20 g) and dimethyl(methylthio)sulfonium tetrafluoroborate (7.0 g, 35.71 mmol) was stirred in dry 1,2-dichloroethane (100 mL) at 0°C for 2 hours. The mixture was neutralized with triethylamine (10 mL), diluted with CH_2Cl_2 (300 mL) and filtered through celite. The filtrate was washed three times with saturated sodium bicarbonate solution (200 mL), dried over $MgSO_4$ and evaporated to dryness. The residue was suspended in diisopropylether (600 mL) and filtered. The resulting solid was crystallized from ethanol (50 ml),

washed with diisopropylether (200 mL) and dried to give 2-[2-(2-thiobenzoyl)ethoxy]ethyl 4,6-O-benzylidene 2-O-(4-chlorobenzoyl)-β-D-galactopyranoside (**19**) (10 g, 66%) as a white powder.

R_f 0.30 (Diethylether/EtOAc 2:1), MS (electrospray) $C_{33}H_{35}ClO_{10}S$ (659.15) m/z (%) 681[M+Na]$^+$ (70), 659[M+H]$^+$ (40)

2-[2-(2-Thiobenzoyl)ethoxy]ethyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-[4,6-O-benzylidene-2,3-di-O-(3,4-methylenedioxybenzyl)]-α-D-galactopyranosyl)-β-D-galactopyranoside (**20**)

A mixture of 2-[2-(2-thiobenzoyl)ethoxy]ethyl 4,6-O-benzylidene 2-O-(4-chlorobenzoyl)-β-D-galactopyranoside (**19**) (8.55 g, 12.99 mmol), methyl 4,6-O-benzylidene-2,3-di-O-(3,4-methylenedioxybenzyl)-1-thio-β-D-galactopyranoside (**18**) (8.00 g, 14.29 mmol), powdered molecular sieves 4A (20 g) and methyl trifluoromethanesulfonate (4.68 g, 28.58 mmol) was stirred in dry 1,2-dichloroethane (100 mL) at room temperature for 2 hours. The mixture was neutralized with triethylamine (4 mL), diluted with CH_2Cl_2 (200 mL) and filtered through celite. The filtrate was washed three times with saturated $NaHCO_3$ solution (200 mL), dried over $MgSO_4$ and evaporated to dryness. The residue was purified by chromatography using diethylether-EtOAc 2:1 as the mobile phase to give 7.5 g of 2-[2-(2-thiobenzoyl)ethoxy]ethyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-[4,6-O-benzylidene-2,3-di-O-(3,4-methylenedioxybenzyl)]-α-D-galactopyranosyl)-β-D-galactopyranoside (**20**) (7.5 g, 50%) as a white solid foam.

R_f 0.55 (Diethylether/EtOAc 2:1), MS (electrospray) $C_{62}H_{61}ClO_{19}S$ (1177.67) m/z (%) 1199[M+Na]$^+$ (100), 1177 (21)

2-[2-(2-Thiobenzoyl)ethoxy]ethyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(4,6-O-benzylidene-α-D-galactopyranosyl)-β-D-galactopyranoside (**21**)

A mixture of 2-[2-(2-thiobenzoyl)ethoxy]ethyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-[4,6-O-benzylidene-2,3-di-O-(3,4-methylenedioxybenzyl)]-α-D-galactopyranosyl)-β-D-galactopyranoside (**20**) (7.02 g, 5.97 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2.71 g, 11.93 mmol) in the mixture of CH_2Cl_2/H_2O 7:2 (70 ml) was stirred at room temperature for 1 hour. The reaction mixture was filtered, the filtrate was diluted with $CHCl_3$ (300 ml), washed twice with saturated $NaHCO_3$ solution (150 ml) and concentrated to dryness. The residue was taken up in hot diisopropylether (150 ml) and the solution was stirred at room temperature for 2 hours. The resulting suspension was filtered, then crystallized from EtOAc (40 ml). The mother liquid was purified by chromatography using diethylether-EtOAc 1:1 mixture as the mobile phase. The purified products were combined to give 2-[2-(2-thiobenzoyl)ethoxy]ethyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(4,6-O-benzylidene-α-D-galacto-pyranosyl)-β-D-galactopyranoside (**21**) (3.69 g, 68%).

R_f 0.32 (Diethylether/EtOAc 2:1), MS (electrospray) $C_{46}H_{45}ClO_{15}S$ (909.40) m/z (%) 931[M+Na]$^+$ (35), 909[M+H]$^+$ (100)

2-[2-(2-Thiobenzoyl)ethoxy]ethyl 2-O-(4-chlorobenzoyl)-3-O-α-D-galactopyranosyl-β-D-galactopyranoside (**22**)

A mixture of 2-[2-(2-thiobenzoyl)ethoxy]ethyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(4,6-O-benzylidene-α-D-galactopyranosyl)-β-D-galactopyranoside (**21**) (3.5 g, 3.85 mmol) and *p*-toluenesulphonic acid (100 mg) in the mixture of acetonitrile-methanol 1:1 (350 ml) was stirred under reflux for 2 hours. The reaction mixture was evaporated to dryness then the residue was chromatographed using MeCN-H_2O 10:1 as the mobile phase to give 2-[2-(2-

thiobenzoyl)ethoxy]ethyl 2-O-(4-chlorobenzoyl)-3-O-α-D-galactopyranosyl-β-D-galactopyranoside (**22**) (2.46 g, 87%).

R_f 0.42 (MeCN/H_2O 10:1), MS (electrospray) $C_{32}H_{41}ClO_{15}S$ (733.13) m/z (%) 755[M+Na]$^+$ (52), 733[M+H]$^+$ (100)

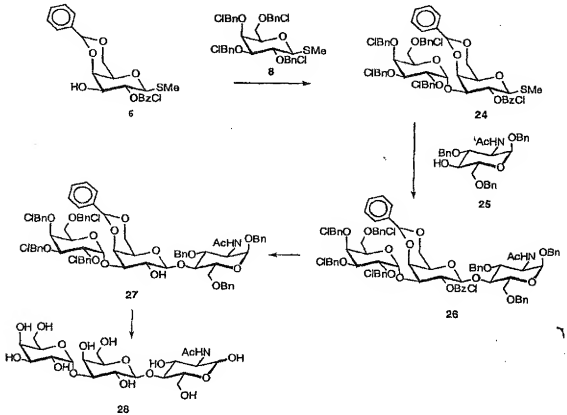
2-[2-(2-Thiobiphenylcarbonyl)ethoxy]ethyl 3-O-α-D-galactopyranosyl-β-D-galactopyranoside (**23**)

A mixture of 2-[2-(2-thiobenzoyl)ethoxy]ethyl 2-O-(4-chlorobenzoyl)-3-O-α-D-galactopyranosyl-β-D-galactopyranoside (**22**) (210 mg, 0.287 mmol) and sodium methoxide (9 mg, 0.287 mmol) in dry methanol (15 ml) was stirred at 40°C for 4 hours. The reaction mixture was cooled to room temperature and biphenylcarbonyl chloride (62.17 mg, 0.287 mmol) was added. After 30 minutes stirring at room temperature, the reaction mixture was evaporated to dryness. The residue was purified by chromatography using MeCN-H_2O 5:1 as the mobile phase to give 2-[2-(2-thiobiphenylcarbonyl)ethoxy]ethyl 3-O-α-D-galactopyranosyl-β-D-galactopyranoside (**23**) (120 mg, 62%).

R_f 0.35 (MeCN/H_2O 10:2), MS (electrospray) $C_{31}H_{42}O_{14}S$ (670.73) m/z (%) 693[M+Na]$^+$ (100), 671[M+H]$^+$ (20)

Example 5: Preparation of 2-Acetamido-2-Deoxy-4-O-[3-O-(α-D-Galactopyranosyl)-β-D-Galactopyranosyl]-D-Glucopyranose (28)

The general strategy for this preparation is set out in Reaction Scheme 4.



Reaction Scheme 4

Methyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-1-thio-β-D-galactopyranoside (**24**)

A mixture of methyl 2,3,4,6-tetra-O-(4-chlorobenzyl)-thio-β-D-galactopyranoside (**8**) (3.9 g, 5.5 mmol), molecular sieves 4Å (4 g) in dry THF (30 ml) was stirred at room temperature, then a solution of bromine (1.18 g , 6.66 mmol) in CH_2Cl_2 (5 ml) was added. The reaction mixture was stirred at room temperature for 10 minutes, then cyclohexene (1 ml) added. To the stirred reaction mixture

methyl 4,6-O-benzylidene 2-O-(4-chlorobenzoyl)-β-D-galactopyranoside (6)(2.0 g, 3.7 mmol)was added then the suspension was cooled to -15°C. A solution of silver trifluoromethanesulphonate (1.4 g, 5.5 mmol) in dry THF (10 ml) was added dropwise under nitrogen atmosphere in 15 minutes. The reaction mixture was kept at 0°C overnight. The reaction mixture was neutralized with triethylamine (2 ml) and filtered. The filtrate was evaporated to dryness and the residue was taken up in $CHCl_3$ (300 mL). The solution was washed with saturated $NaHCO_3$ solution (3 x 300 mL). The organic phase was dried over $MgSO_4$ and evaporated to dryness to give an oily residue. The residue was chromatographed using diethylether-ethanol 20:1 as the mobile phase to give methyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(2,3,4,6-tetra-O-(4-chloro-benzyl)-α-D-galactopyranosyl)-1-thio-β-D-galactopyranoside (**24**) (1.60 g, 40%).

R_f 0.30 (Diethylether), MS (electrospray) $C_{55}H_{51}Cl_5O_{11}S$ (1097.33) m/z (%) 1117$[M+Na]^+$ (100), 1095$[M+H]^+$ (32)

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-α-D-glucopyranoside (**26**)

A mixture of methyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-1-thio-β-D-galactopyranoside (**24**) (430 mg, 0.39 mmol), benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside (**25**) (300 mg, 0.59 mmol), molecular sieves 4Å (5 g) and methyl trifluoromethane-sulphonate (97 mg, 0.59 mmol) in dry 1,2-dichloroethane (15 ml) was stirred at room temperature overnight. The reaction mixture was neutralized with triethylamine (2 ml) and filtered. The filtrate was diluted with $CHCl_3$ (100 ml) and was washed with saturated $NaHCO_3$ solution (2 x 100 mL). The organic phase was dried over $MgSO_4$ and evaporated to

dryness to give an oily residue. The residue was chromatographed using diethylether-ethanol 25:1 as the mobile phase to give benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-α-D-glucopyranoside (**26**) (300 mg, 50%).

R_f 0.33 (Diethylether/EtOH 25:1), MS (electrospray) $C_{83}H_{80}Cl_5NO_{17}$ (1540.83) m/z (%) 1560[M+Na]$^+$ (100), 1538[M+H]$^+$ (27)

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-α-D-glucopyranoside (**27**)

To a solution of sodium methoxide (73 mg, 0.13 mmol) in dry methanol (10 mL), benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-α-D-glucopyranoside (**26**) (300 mg, 0.19 mmol was added. The resulting mixture was stirred at 40°C for 4.5 hours. The reaction mixture was kept at 0°C for 1 hour and filtered. The solid precipitate was washed with cold dry MeOH (10 ml) to give benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-α-D-glucopyranoside (**27**) (190 mg, 67%) as a white powder.

R_f 0.35 ($CHCl_3$/MeOH 7:3), MS (electrospray) $C_{76}H_{77}Cl_4NO_{16}$ (1402.27) m/z (%) 1423[M+Na]$^+$ (100), 1401[M+H]$^+$ (35)

2-Acetamido-2-deoxy-4-O-[3-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-D-glucopyranose (**28**)

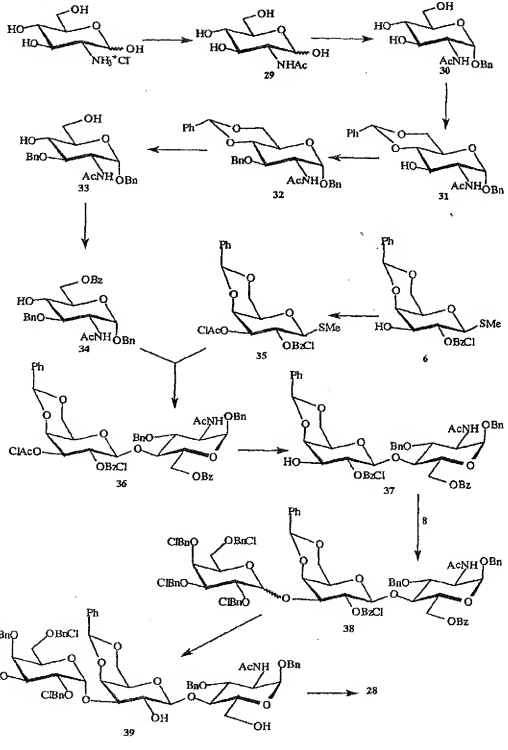
To a suspension of Pd/C (10%) catalyst (1.0 g), benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[4,6-O-

benzylidene-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-α-D-glucopyranoside (**27**) (190 mg, 0.13 mmol) and acetic acid (3 drops) was shaken under a positive pressure (45 PSI) of hydrogen for 4 hours. The reaction mixture was filtered through celite and the filtrate was neutralized (pH 7.0) with excess mixed bed resin (Amberlite-MB 1).The resin was filtered off and the filtrate was evaporated to dryness. The residue was taken up in milli-Q water (10 mL) and the resulting solution was filtered using a 0.22 µm filter. The filtrate was passed through a C-18 Sep-pak cartridge (1 g). The filtrate was evaporated to dryness and the remaining solid was further dried over phosphorus pentoxide at room temperature under high vacuum to give 2-acetamido-2-deoxy-4-O-[3-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-D-glucopyranose (**28**) (32 mg, 43%) as a white solid.

R_f 0.36 ($CHCl_3$/MeOH/H_2O 10:12:3), MS (electrospray) $C_{20}H_{35}NO_{16}$ (545.50) m/z (%) 568[M+Na]$^+$ (100), 546[M+H]$^+$ (52)

*Example 6: Alternative Synthesis of Compound (**28**)*

Compound (**28**) may also be prepared using a different glucosamine acceptor, benzyl-6-0-benzoyl-3-0-benzoyl 1-2-acetamido-2-deoxy-x-D-glucopyranoside, using the strategy set out in Reaction Scheme 5. The acceptor can readily be prepared in high yield.



Reaction Scheme 5

2-Acetamido-2-deoxy-D-glucopyranose (29)

Sodium (23.4g, 1.02 mol) was reacted with dry methanol (1.6 L), then the resulting solution was cooled to 40 °C. Glucosamine hydrochloride (200 g, 0.926 mol) was added to the solution and the reaction mixture was stirred vigorously for 5 minutes. The suspension was filtered in dry conditions. Acetic anhydride (140 mL, 1.48 mol) was added dropwise to the filtrate at 0 °C in 30 min. The resulting suspension was stirred at room temperature for another 30 minutes. The reaction mixture was diluted with ether (2 L), filtered and the solid product was dried to give 2-acetamido-2-deoxy-D-glucopyranose (29)(177 g, 86 %).

Benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside (30)

A mixture of 2-acetamido-2-deoxy-D-glucopyranose (29) (150 g, 0.68 mol), Amberlite IR 120 [H^+] ion exchange resin (150 g) in benzyl alcohol (1.25 L) was stirred at 80 °C for 3.5 hours. The reaction mixture was filtered. The filtrate was evaporated under reduced pressure at 90 C°. The residue was taken up in hot isopropanol (600 mL) and filtered. The filtrate was left to crystallize, the white crystalline solid was filtered off, washed twice with cold isopropanol (200 mL) and twice with ether (200 mL) to give 2-acetamido-2-deoxy-α-D-glucopyranoside (30)(56.2 g, 27%).

Benzyl 4,6-O-benzylidene-2-acetamido-2-deoxy-α-D-glucopyranoside (31)

Benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside (30) (50 g, 0.16 mmol) was dissolved in dry DMF (200 mL). Dry acetonitrile (100 mL), α,α-dimethoxytoluene (29 g, 0.19 mol, 1.2 eq) and p-toluenesulphonic acid (50 mg) was added to the DMF solution. The reaction mixture was stirred at 80 °C for 2 hours under vacuum (350 mbar); the product started to precipitate after 1 hour. The resulting suspension was cooled (60 °C) and the pH adjusted to 7 by addition of triethylamine. The suspension was cooled to 0 °C, and cold methanol (500 mL) (-10 °C) was added slowly to the mixture.

The product was filtered, washed with cold methanol (200 mL) then with cold ether (2 x 200 mL) to give benzyl 4,6-O-benzylidene-2-acetamido-2-deoxy-α-D-glucopyranoside (**31**) (48 g, 75 %).

Benzyl 3-O-benzyl-4,6-O-benzylidene-2-acetamido-2-deoxy-α-D-glucopyranoside (**32**)

A suspension of sodium hydride (3.6 g, 0.15 mol, 1.2 eq) in dry DMF (25 mL) was cooled to 0 °C, and a solution of benzyl 4,6-O-benzylidene-2-acetamido-2-deoxy-α-D-glucopyranoside (**32**) (50 g, 0.125 mol) in dry DMF (450 mL) was added dropwise in 30 minutes. The resulting solution was stirred at 0 °C for 30 minutes and benzyl bromide was added (25.66 g, 0.15 mol, 1.2 eq) dropwise at 0 °C (the product started to precipitate at the beginning of the addition of the benzyl bromide). The reaction mixture was stirred at room temperature for 45 minutes, cooled to 0 °C and dry methanol (25 mL) was added dropwise. The reaction mixture was diluted with cold ether (1 L) and the mixture was stirred for 30 minutes. The resulting suspension was filtered and washed three times with ether (400 mL) to give benzyl 3-O-benzyl-4,6-O-benzylidene-2-acetamido-2-deoxy-α-D-glucopyranoside (**32**) (62.0 g) as a white powder with quantitative yield.

Benzyl 3-O-benzyl-2-acetamido-2-deoxy-α-D-glucopyranoside (**33**)

A suspension of benzyl 3-O-benzyl-4,6-O-benzylidene-2-acetamido-2-deoxy-α-D-glucopyranoside (**32**) (50 g, 0.102 mol) in acetic acid (500 mL) and water (25 mL) was stirred at 110 °C for 45 minutes. The reaction mixture was concentrated under reduced pressure at 40 C°. The oily residue was taken up twice in toluene (200 mL) and concentrated. The residue was treated with di-isopropyl ether (250 mL) and the resulting suspension was strirred for 30 minutes. The white solid was filtered off, washed twice with cold ether (200 mL) to give benzyl 3-O-benzyl-2-

acetamido-2-deoxy-α-D-glucopyranoside (**33**) (38.0 g, 93%).

Benzyl 6-O-benzoyl-3-O-benzyl-2-acetamido-2-deoxy-α-D-glucopyranoside (**34**)

A solution of benzoyl chloride (6.3g, 0.045 mol, 1.2eq) and imidazole (6 g, 0.09mol, 2.4 eq) in dry 1,2-dichloroethane (150 mL) was stirred for 20 minutes at 5 °C. The resulting suspension was filtered under dry conditions. The filtrate was added to a solution of benzyl 3-O-benzyl-2-acetamido-2-deoxy-α-D-glucopyranoside (**33**) (15g, 37.6 mmol) in dry 1,2-dichloroethane (600 mL). The reaction mixture was stirred at 90 °C for 48 hours and cooled to room temperature. The resulting suspension was filtered, washed twice with brine (300 mL), dried over $MgSO_4$ and concentrated. The residue was taken up in hot isopropanol (300 mL) and left to crystallize. The white crystalline solid was filtered off to give Benzyl 6-O-benzoyl-3-O-benzyl-2-acetamido-2-deoxy-α-D-glucopyranoside (**34**) (11.7 g, 62%).

Methyl 4,6-O-benzylidene-3-O-chloroacetyl-2-O-(4-chlorobenzoyl)-1-thio-β-D-galactopyranoside (**35**)

A mixture of methyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-1-thio-β-D-galactopyranoside (**6**) (10.0 g, 23 mmol) and 4-dimethylaminopyridine (3.40 g, 27.8 mmol) in dry 1,2-dichloroethane (100 mL) was stirred at 0 °C, then chloroacetyl chloride (3.4 g , 27.8 mmol, 1.2 eq) was added dropwise to the solution. The reaction mixture was stirred at room temperature for 2.5 hours, then diluted with 1,2-dichloroethane (100 mL). The resulting solution was washed twice with saturated brine solution (100 ml), dried over $MgSO_4$ and concentrated to give methyl 4,6-O-benzylidene-3-O-chloroacetyl-2-O-(4-chlorobenzoyl)-1-thio-β-D-galactopyranoside (**35**) (10.2 g, 86%) as a white crystalline solid.

Benzyl 2-acetamido-6-O-benzoyl-3-O-benzyl-4-O-[4,6-O-benzylidene-3-O-chloroacetyl-2-O-(4-chlorobenzoyl)-β-D-galactopyranosyl]-2-deoxy-α-D-glucopyranoside (**36**)

To a mixture of benzyl 2-acetamido-6-O-benzoyl-3-O-benzyl-4-O-2-deoxy-α-D-glucopyranoside (**34**) (5 g, 9.9 mmol), methyl 4,6-O-benzylidene-3-O-chloroacetyl-2-O-(4-chlorobenzoyl)-1-thio-β-D-galactopyranoside (**35**) (5.71 g, 11.1 mmol, 1.12 eq) and Molecular sieves 4A (2.5 g) in dry 1,2-dichloroethane (300 mL), DMTST (5.75g, 2.4 eq) was added under nitrogen. The reaction mixture was stirred at room temperature for 5 hours, then neutralized by addition of pyridine (5 mL). Acetic anhydride was added (2.5 mL) and the reaction mixture was stirred at room temperature for 0.5 hours. The resulting suspension was filtered through a bed of Celite. The filtrate was washed with a saturated solution of $NaHCO_3$ (200 mL), twice with brine (200 ml), dried over $MgSO_4$ and concentrated. The residue was taken up in DCM (25 mL) and diisopropyl ether (200 mL) was added. The resulting yellow precipitate was filtered off and washed twice with cold diisopropyl ether (100 mL). The solid was crystallized using a mixture of DCM (20 mL) and ether (25 mL) to give benzyl 2-acetamido-6-O-benzoyl-3-O-benzyl-4-O-[4,6-O-benzylidene-3-O-chloroacetyl-2-O-(4-chlorobenzoyl)-β-D-galactopyranosyl]-2-deoxy-α-D-glucopyranoside (**36**) (5.1 g, 55%) as a white crystalline solid.

Benzyl 2-acetamido-6-O-benzoyl-3-O-benzyl-4-O-[4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-β-D-galactopyranosyl]-2-deoxy-α-D-glucopyranoside (**37**)

A mixture of benzyl 2-acetamido-6-O-benzoyl-3-O-benzyl-4-O-[4,6-O-benzylidene-3-O-chloroacetyl-2-O-(4-chlorobenzoyl)-β-D-galactopyranosyl]-2-deoxy-α-D-glucopyranoside (**36**) (0.5 g) and thiourea (303 mg) in THF (3 mL) and water (0.5 mL) was stirred at room temperature for 14 hours, then the reaction mixture was diluted with chloroform (100 mL). The resulting solution was washed

twice with water (50 ml), dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography using DCM / EtOAc 1:1 as the mobile phase to give benzyl 2-acetamido-6-O-benzoyl-3-O-benzyl-4-O-[4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-β-D-galactopyranosyl]-2-deoxy-α-D-glucopyranoside (**37**) (280 mg, 61 %) as a white solid.

Benzyl 2-acetamido-6-O-benzoyl-3-O-benzyl-2-deoxy-4-O-[4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α,β-D-galactopyranosyl)-β-D-galactopyranosyl)]-α-D-glucopyranoside (**38**)

To a mixture of methyl 2,3,4,6-tetra-O-(4-chlorobenzyl)-1-thio-β-D-galactopyranoside (430 mg, 0.602 mmol), benzyl 2-acetamido-6-O-benzoyl-3-O-benzyl-4-O-[4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-β-D-galactopyranosyl]-2-deoxy-α-D-glucopyranoside (**37**) (280 mg, 0.301 mmol) and molecular sieves 4Å (300 mg) in dry 1,2-dichloroethane (3 mL), DMTST (300 mg, 1.2 mmol) was added. The reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was neutralized with triethylamine (1 ml), diluted with $CHCl_3$ (50 mL) and filtered. The filtrate was then washed with saturated $NaHCO_3$ solution (3 x 50 mL). The organic phase was dried over $MgSO_4$ and evaporated to dryness to give a solid foam. The residue was purified by chromatography using $CHCl_3$ - EtOAc 1:1 as the mobile phase to give benzyl 2-acetamido-6-O-benzoyl-3-O-benzyl-2-deoxy-4-O-[4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α,β-D-galactopyranosyl)-β-D-galactopyranosyl)]-α-D-glucopyranoside (**38**) (325 mg, 70%, α/β = 85/15).

Benzyl 2-acetamido-3-O-benzyl-2-deoxy-4-O-[4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-α-D-glucopyranoside (**39**)

To a solution of sodium methoxide (20 mg, 0.37 mmol) in dry methanol (2 mL), benzyl 2-acetamido-6-O-benzoyl-3-O-benzyl-2-deoxy-4-O-[4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α,β-D-galactopyranosyl)-β-D-galactopyranosyl)]-α-D-glucopyranoside (**38**) (190 mg, 0.12 mmol was added. The resulting mixture was stirred at 40°C for 4 hours. The reaction mixture was cooled to room temperature and filtered. The solid precipitate was washed with cold dry MeOH (10 mL), followed by hexane (2 x 25 mL) to give benzyl 2-acetamido-3-O-benzyl-2-deoxy-4-O-[4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-α-D-glucopyranoside (**39**) (110 mg, 68%) as a white powder. TLC R_f 0.35 ($EtOAc/CHCl_3$ 7:3

2-Acetamido-2-deoxy-4-O-[3-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-D-glucopyranose (**28**)

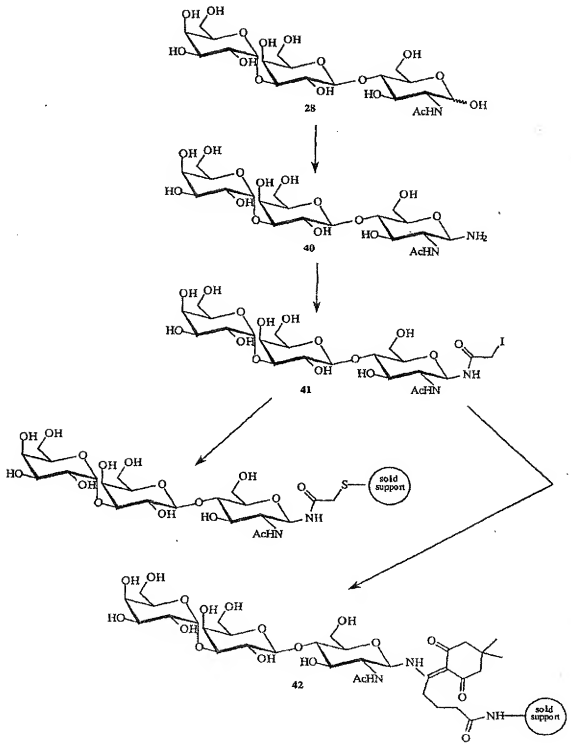
To a suspension of Pd/C (10%) catalyst (100 mg), benzyl 2-acetamido-3-O-benzyl-2-deoxy-4-O-[4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-α-D-glucopyranoside (**39**) (80 mg, 0.06 mmol) and acetic acid (3 drops) in THF-MeOH-H2O 5:1:1 (7 mL) was shaken under a positive pressure (60 PSI) of hydrogen overnight. The reaction mixture was diluted with milliQ water (30 mL), filtered through Celite and the filtrate was neutralized (pH 7.0) with excess mixed bed resin (Amberlite-MB 1). The resin was filtered off and the filtrate was evaporated to dryness. The residue was taken up in milli-Q water (5 mL) and the resulting solution was passed through a C-18 Sep-pak cartridge (1 g). The filtrate was evaporated to dryness and the remaining solid was further dried over phosphorus pentoxide at room temperature under high vacuum to give 2-

acetamido-2-deoxy-4-O-[3-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-D-glucopyranose (**28**) (20 mg, 53%) as a white solid.

R_f 0.36 ($CHCl_3$/MeOH/H_2O 10:12:3), MS (electrospray) $C_{20}H_{35}NO_{16}$ (545.50) m/z (%). 568[M+Na]$^+$ (100), 546[M+H]$^+$ (52)

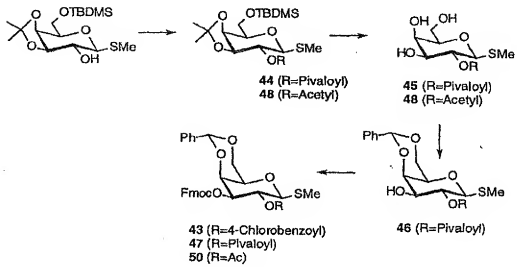
Example 6: Immobilization of 2-acetamido-2-deoxy-4-O-[3-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-D-glucopyranose (28)

The following reaction scheme, Scheme 6, illustrates how a compound of the invention can be bound to a solid support, using two alternative linking groups. The second linking group is a dioxo compound, as discussed in our International patent application No. PCT/AU98/00808. It will be appreciated that other compounds of the invention can be linked to a solid support in a similar manner.



Scheme 6

Scheme 7: Synthesis of Participating Galactopyranoside Building Blocks



Scheme 7

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Example 7: Synthesis of Methyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-fluorenylmethyl-oxycarbonyl-1-thio-β-D-galactopyranoside

10 *Methyl 4,6-O-benzylidene-2-O-(4-chlorobenzoyl)-3-O-fluorenylmethyloxycarbonyl-1-thio-β-D-galactopyranoside* (**43**)

A suspension of methyl 4,6-*O*-benzylidene-2-*O*-(4-chlorobenzoyl)-1-thio-β-D-galactopyranoside *6* [20g, 45.87mmol] in 1,2-dichloroethane [200mL] was cooled to 0°C. To the cooled suspension was added DMAP [16.81g, 138mmol] followed by

15 Fmoc-Cl [35.60g, 137mmol]]. The now solution was returned to ambient temperature and stirred for 2 hours. The reaction mixture was then diluted with Chloroform [200mL] and washed with 5% citric acid solution [2 x 400mL] and saturated brine solution [2 x 400mL]. The layers were separated and the organic layer dried over Na_2SO_4 followed by filtration and removal of the solvent *in vacuo*. The resulting residue was purified by

20 column chromatography [20% ethylacetate/petroleum ethers v/v] to afford methyl 4,6-*O*-benzylidene-2-*O*-(4-chlorobenzoyl)-3-*O*-fluorenylmethyloxycarbonyl-1-thio-β-D-galactopyranoside 43 as a white foam [27.2g, 90%]; $R_f = 0.22$; ES-MS gave *m/z* (ion, relative intensity); ^1H NMR ($CDCl_3$) δ 7.88-7.07 (17H, aromatic), 6.01 (t, 1H, H-2), 5.79 (s, 1H, benzylidene) 5.36 (dd, 1H, H-3), 4.91 (d, 1H, $J_{1,2}$=8.5, H-1), 4.89 (d, 1H, H-

4), 4.78 (dd, 1H, H-6_b), 4.67 (m, 2H, Fluorenyl-CH_2-), 4.52 (t, 1H, 9-fluorenyl-*methyne*), 4.49 (dd, 1H, H-6_b), 4.14 (s, 1H, H-5), 2.29 (s, 3H, S-CH_3)

Example 8: Synthesis of Methyl 4,6-O-benzylidene-3-O-fluorenylmethyloxycarbonyl-2-O-pivaloyl-1-thio-β-D-galactopyranoside

Methyl 6-O-tert-butyldimethylsilyl)-3,4-O-isopropylidene-2-O-(pivaloyl)-1-thio-β-D-galactopyranoside (44)

To a mixture 6-*O*-tert-butyldimethylsilyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside [11.5g, 31.59mmol] and DMAP [5.5g, 45.5mmol] in 1,2-dichloroethane [40mL] was added dropwise, 2,2,2-trimethylacetylchloride. The reaction was stirred for 2 hours then diluted with chloroform [100mL] and washed with 10% citric acid solution [2 x 150mL], saturated $NaHCO_3$ solution [2 x 150mL] and saturated brine solution [2 x 150mL]. The layers were separated and the organic layer dried over Na_2SO_4. The solvent was removed *in vacuo* to give an oily residue. The residue was purified by column chromatography (5%ethylacetate/petroleum ethers) to give a white foam, methyl 6-*O*-tert-butyldimethylsilyl-3,4-*O*-isopropylidene-2-*O*-pivaloyl-1-thio-β-D-galactopyranoside **44** [13.7g, 97%]. R_f = 0.75 (ethylacetate/petroleum ethers, 1:2, v/v); ^1H NMR ($CDCl_3$) δ 5.05 (dd, 1H, H-2), 4.29 (dd, 1H, H-4), 4.25 (d, 1H, J_{1-2}=10.12, H-1), 4.17 (dd, 1H, H-3), 3.93-3.84 (m, 3H, H-6_a, H-6_b, H-5), 2.16 (s, 3H, S-CH_3),

Methyl 2-O-pivaloyl-1-thio-β-D-galactopyranoside (45)

Methyl 6-*O*-tert-butyldimethylsilyl-2-*O*-pivaloyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside **44** [3.34g, 7.45mmol] x, was dissolved in 25% acetonitrile/methanol [40mL]. To the solution was added 4-toluenesulphonic acid [17mg, 90.43μmol], the solution was then stirred under refluxed for 3 hours. The reaction temperature was then reduced to 40°C and left overnight. The reaction mixture was then concentrated and the residue azeotroped with

toluene followed by diethylether to give a white residue. The residue was purified by column chromatography (10% acetonitrile/ethylacetate, v/v) to give a white solid, methyl 2-*O*-pivaloyl-1-thio-β-*D*-galactopyranoside **45** [2.19g, 83%], R_f = 0.20 (ethylacetate); ES-MS *m/z* (ion, relative intensity) 295 ($[M+H]^+$, 100%); ^1H NMR ($CDCl_3$) δ5.08 (dd, 1H, H-2), 4.39 (d, 1H, J_{1-2} = 9.88Hz, H-1), 4.13 (d, 1H, H-4), 4.01-3.92 (m, 2H, H-6_a, H-6_b), 3.72 (dd, 1H, H-3), 3.62 (dd, 1H, H-5), 2.21 (s, 3H, S-CH_3), 1.27 (s, 9H, *t*-butyl).

Methyl 4,6-O-benzylidene-2-O-pivaloyl-1-thio-β-D-galactopyranoside (**46**)

A mixture of methyl 2-*O*-(pivaloyl)-1-thio-β-D-galactopyranoside **45** [1.68g, 5.71mmol], α,α-dimethoxytoluene and 4-toluenesulphonic acid [10mg, 43.19mmol] was dissolved in acetonitrile [50mL] and heated at 60°C with stirring for 1 hour. The reaction was then allowed to return to ambient temperature, neutralised with 2 equivalents of triethylamine and concentrated under vacuum. The residue was taken up in chloroform [100mL] and the organic layer washed with dilute brine [3:1; H_2O:Brine, 1 x 100mL], saturated $NaHCO_3$ solution [1 x 100mL], and saturated brine solution [1 x 100mL]. The layers were separated and the organic layer dried over Na_2SO_4. The organic layer was concentrated and the residue purified by column chromatography (33% ethylacetate/petroleum ethers, v/v) to give methyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-1-thio-β-D-galactopyranoside **46** [1.91g, 87%]. R_f = 0.63 (ethylacetate), ES-MS *m/z* (ion, relative intensity) 341 ($[M+H]^+$, 100%); ^1H NMR ($CDCl_3$) δ 7.51 (m, 2H, aromatic) 7.41 (m, 3H, aromatic), 5.58 (s, 1H, C*H*-benzylidene), 5.24 (dd, 1H, H-2), 4.4 (dd, 1H, H-6_a), 4.39 (d, 1H, J_{1-2} = 9.77, H-1), 4.29 (dd, 1H, H-4), 4.08 (dd, 1H, H-6_b), 3.8 (ddd, 1H, H-3), 3.60 (s, 1H, H-5), 2.26 (s, 3H, S-CH_3), 1.27 (s, 9H, *t*-butyl)

Methyl 4,6-O-benzylidene-3-O-fluorenylmethyloxycarbonyl-2-O-pivaloyl-1-thio-β-D-galactopyranoside (**47**)
Methyl 4,6-*O*-benzylidene-2-*O*-pivaloyl-1-thio-β-D-galactopyranoside **46** [1.90g, 4.97mmol] was dissolved in 1,2-dichloroethane (20mL) and the resulting solution was cooled to 0°C. At this time DMAP [1.82g, 14.92mmol] and Fmoc-Cl [3.87g, 14.92mmol] were added sequentially. The cold bath was then removed, and the reaction allowed to return to room temperature. The reaction was stirred at ambient temperature for 2 hours and then diluted with $CHCl_3$ [~50mL]. The reaction mixture was then washed with 5% citric acid solution [2 x 100mL] and saturated brine solution [2 x 100mL]. The layers were separated and the organic layer dried over Na_2SO_4. The solution was then filtered and concentrated to afford a yellow residue which was purified by column chromatography (20% ethylacetate/petroleum ethers v/v) to give methyl 4,6-*O*-benzylidene-3-*O*-fluorenylmethyloxycarbonyl-2-*O*-pivaloyl-1-thio-β-D-galactopyranoside **47** [2.74g, 91%]; R_f = 0.38 (25% ethylacetate/petroleum ethers v/v); ES-MS *m/z* (ion, intensity); ^1H NMR ($CDCl_3$) δ 7.78-7.25 (13H, aromatic), 5.61 (t, 1H, H-2), 5.57 (s, 1H, benzylidene), 4.97 (dd, 1H, H-3), 4.50 (d, 1H, H-4), 4.45 (d, 1H, J_{1-2}=9.10hz, H-1), 4.47-4.33 (m, 2H, Fmoc-CH_2-), 4.25 (t, 1H, 9-fluorenyl-*methyne*), 4.40, (dd, 1H, H-6_a) 4.08 (dd, 1H, H-6_b) 3.65 (s, 1H, H-5), 2.30 (s, 3H, S-CH_3), 1.20 (s, 9H, *t*-butyl)

Example 9: Synthesis of Synthesis of methyl 2-O-acetyl-4,6-O-benzylidene-3-O-fluorenylmethyloxycarbonyl-1-thio-β-D-galactopyranoside

Synthesis of methyl 2-O-acetyl-6-O-tert-butyldimethylsilyl-3,4-O-isopropylidene-1-thio-β-D-galactopyranoside (**48**)

A mixture of methyl 6-*O*-tert-butyldimethylsilyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (3.00g, 8.24mmol) and 4-dimethylaminopyridine (2.42g, 19.78mmol) in dry 1,2-dichloroethane (750 ml) was stirred at room temperature. Acetyl chloride [1.05mL, 14.84mmol] was added dropwise to the solution over 15 minutes. The reaction stirred at room temperature for 2 hours at which time it was diluted with chloroform and washed with 10% citric acid solution [2 x 100mL] saturated sodium hydrogen carbonate [2 x 100mL] and finally with saturated brine solution [2 x 100mL]. The layers were separated and the organic layer dried over Na_2SO_4. The solution was filtered and concentrated to afford a white residue which was purified by column chromatography (20% ethylacetate/petroleum ethers v/v) to afford methyl 2-*O*-acetyl-6-*O*-tert-butyldimethylsilyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside **48** as a white solid [2.65g, 79%]; R_f = 0.43 (25% ethylacetate/petroleum ethers v/v)

Synthesis of methyl 2-O-acetyl-1-thio-β-D-galactopyranoside (**49**)

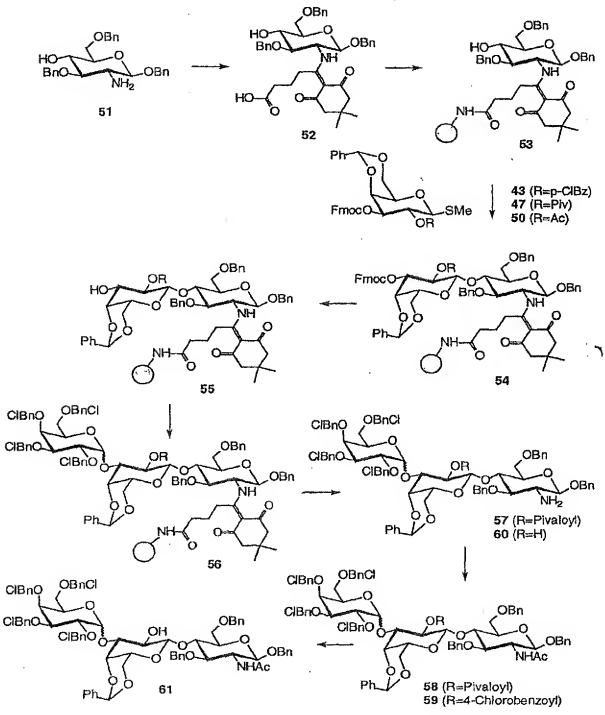
2-*O*-Acetyl-6-*O*-tert-butyldimethylsilyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside x was dissolved in 50% acetonitrile/methanol [50mL] and heated at 60°C. To the stirred solution was added 4-toluenesulphonic acid [10.mg, 53.19μmol] and the reaction was left for 4 hours. The reaction temperature was then reduced to 40°C and left overnight. The reaction mixture was then concentrated and the residue crystallised from methanol to afford 2-*O*-acetyl-1-thio-β-D-galactopyranoside **49** as a white solid

[1.26g, 79%]; R_f = 0.2 (25% acetonitrile/ethylacetate, v/v); ^{1}H NMR (*d*-MeOH) δ 3.95 (t, 1H, H-2), 3.27 (d, 1H, J_{1-2}=8.63, H-1), 2.92, 1H, H-4), 2.79-2.69 (m, 2H, H-6_a and H-6_b), 2.62 (t, 1H, H-3), 2.38 (m, 1H, H-5) 1.37 (s, 3H, S-CH_3), 1.31 (s, 3H, -C(O)CH_3)

Synthesis of methyl 2-O-acetyl-4,6-O-benzylidene-3-O-fluorenylmethyloxycarbonyl-1-thio-β-D-galactopyranoside **(50)**

2-*O*-Acetyl-1-thio-β-D-galactopyranoside **49** was dissolved in acetonitrile [20mL] and heated to 60°C. To the stirred solution was added α,α-dimethoxytoluene [1.09g, 7.10mmol] and 4-toluenesulphonic acid [10mg, 53.19μmol]. The reaction was stirred for 2 hours and then allowed to return to room temperature. The reaction was neutralised with 2 equivalents of triethylamine and evaporated to dryness. The residue was taken up in chloroform and washed with dilute brine [1 x 100mL], saturated sodium hydrogencarbonate solution [1 x 100mL] and saturated brine solution [1 x 100mL]. The layers were separated and the organic layer dried over Na_2SO_4. The solution was filtered and concentrated. The residue was washed successively with petroleum ethers, and the resulting white solid then suspended in toluene and any remaining water azeotroped under co-evaporation. The residue from the previous step was suspended in 1,2-dichloroethane [20mL] and cooled to 0°C. To the stirred suspension at 0°C was added 4,4-dimethylaminopyridine [1.62g, 13.23mmol] and Fmoc-Cl [3.42g, 12.23mmol]. The now solution was allowed to return to room temperature and stirred for 1 hour. At this time the reaction was diluted with chloroform and washed with 5% citric acid solution [2 x 75mL] and saturated brine solution [2 x 75mL]. The layers were then separated and the organic layer dried over Na_2SO_4. The solution was filtered and the solvent removed *in vacuo* to give a yellow oily residue which was purified by column chromatography (33% ethylacetate/petroleum ethers v/v) to give methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-fluorenylmethyloxycarbonyl-1-thio-β-D-galactopyranoside **50** [2.19g, 82%] R_f = 0.2 (33% ethylacetate/petroleum ethers, v/v); ^{1}H NMR ($CDCl_3$) δ 7.78-7.24 (13H, aromatic), 5.60 (t, 1H, H-2), 5.55 (s, 1H, benzylidene), 4.88 (dd, 1H, H-2), 4.50 (d, 1H, H-4), 4.55-4.38 (m, 4H, H-1, Fmoc-CH_2, H-6_a), 4.28 (t, 1H, 9-fluorenyl-*methyne*), 4.06 (dd, 1H, H-6_b), 3.63 (s, 1H, H-5), 2.29 (s, 3H, S-CH_3), 2.1 (s, 3H, -C(O)CH_3)

Scheme 8: Solid Phase Synthesis of Galα(1-3)-β(1-4)-GlcNAc



Example 10: Synthesis of a partially protected resin-linker-sugar conjugate.

Benzyl 3,6-di-O-benzyl-2-deoxy-2-amino-β-D-glucopyranoside **(51)**

To a solution benzyl of 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside [6.20g, 10.71mmol] in ethanol [100mL], was added hydrazine hydrate [6.2mL, 55%/H_2O] and water [5mL]. The solution was refluxed overnight and then allowed to return to ambient temperature. The solution was filtered, the solvent removed *in vacuo*, and the residue taken up in $CHCl_3$ [200mL]. The Chloroform suspension was filtered, the filtrate dried over Na_2SO_4 and concentrated under reduced pressure to give a pure clear oil, benzyl 3,6-Di-*O*-benzyl-2-deoxy-2-amino-β-D-glucopyranoside **51** [4.7g, 97%]; R_f = 0.5 (Acetonitrile), ES-MS gave *m/z* (ion, relative intensity): 450 ($[M+H]^+$, 100%); ^1H NMR ($CDCl_3$) δ 7.43-7.30 (m 15H, aromatic), 5.00-4.60 (6H, $3CH_2$-C_6H_5), 4.38 (d, 1H, $J_{1,2}$ = 7.92Hz, H-1), 3.85-3.75 (m, 3H, H-6a, H-6b, H-3), 3.53 (ddd, 1H, H-5), 3.38 (dd, 1H, H-3), 2.92 (dd, 1H, H-2).

Benzyl 3,6-Di-O-benzyl-2-deoxy-2-N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-pentanoic acid-6-yl)-β-D-glucopyranoside **(52)**

To a solution of Benzyl 3,6-Di-*O*-benzyl-2-deoxy-2-amino-β-D-glucopyranoside **51** [4.70g, 10.47mmol] in ethanol [100mL], was added 6-hydroxy-6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-pentanoic acid [5.32g, 20.93mmol] followed by the addition of triethylamine [1.5mL, 10.69mmol]. The reaction mixture was heated overnight at 60°C and then allowed to return to room temperature. The reaction mixture was concentrated and the residue taken up in chloroform [200mL]. The organic layer was washed with a solution of 0.3*N* HCl [2 x 200mL] and saturated Brine solution [1 x 200mL]. The organic layer was dried over

Na_2SO_4 and concentrated to give a pale yellow residue. The residue was purified by column chromatography with ethylacetate-petroleum ethers-acetic acid, 5:15:0.4 to give benzyl 3,6-Di-*O*-benzyl-2-deoxy-2-N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-pentanoic acid-6-yl)-β-D-glucopyranoside **52** [6.09g, 85%]. R_f = 0.10 (ethylacetate-petroleum ethers-acetic acid, 5:15:0.4), ES-MS *m/z* (ion, relative intensity): 686.5 ($[M+H]^+$, 100%)

Coupling of Benzyl 3,6-Di-O-benzyl-2-deoxy-2-N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-pentanoic acid-6-yl)-β-D-glucopyranoside to MBHA resin (0.7mmol/g) (**53**)

In a 200mL peptide reaction vessel MBHA resin [11.86g, 8.30 mmol] was swollen in a minimum of dry *N,N*-dimethylformamide (DMF). A DMF [50mL] solution was made of Benzyl 3,6-Di-*O*-benzyl-2-deoxy-2-N-(6-(4,4-dimethyl-2,6-dioxocyclohexylidene)-pentanoic acid-6-yl)-β-D-glucopyranoside **52** [6.09g, 8.90 x mmol], diisopropylethylamine (DIPEA) [3.11mL, 17.8mmol] and *O*-Benzotriazole-1-yl-*N,N,N',N'*-tetramethyluroniumhexa-fluorophosphate (HBTU) [3.37g, 8.9mmol] which was then added to the reaction vessel. The vessel was sealed and shaken overnight. Ninhydrin assay indicated that the reaction was greater than 99.4% complete, the reaction was stopped, and the resin was washed with DMF [4 x 100mL], 50% DCM/MeOH [4 x 100mL] and DCM [4 x 100mL]. The resin was dried under house vacuum for 4 hours and then dried under high vacuum overnight. Yield of resin **53** was [17.15g, 98.6% by weight].

Synthesis of benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[4,6-O-benzylidene-2-O-pivaloyl-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-β-D-glucopyranoside (**58**)

Under an atmosphere of nitrogen, resin **53** [300mg, 141μmol], 4,6-*O*-benzylidene-3-*O*-fluorenylmethyloxycarbonyl-2-*O*-pivaloyl-1-thio-β-D-galactopyranoside **47** [557mg, 846μmol] and powdered molecular sieves 4Å [600mg], were

suspended in dichloromethane [3mL], followed by the addition of methyl trifluoromethanesulphonate [95.7µL, 846µmol]. The reaction vessel was sealed and the reaction mixture agitated for five hours at ambient temperature. The resin was then washed with DMF [3 x 20mL], 50% MeOH/DCM [3 x 20mL] and DCM [3 x 20mL]. The resin was then floated in DCM to separate the resin from any remaining sieves. Resin **54** was collected and dried under house vacuum for 1 hour. The resin was then treated with a 20% triethylamine/DMF solution for 25mins followed by workup as above. Resin **55** was dried under hi-vacuum overnight. Under an atmosphere of nitrogen the resin was then combined with methyl 2,3,4,6-tetra-*O*-(4-chlorobenzyl)-1-thio-β-D-galactopyranoside **8** [600mg, 846µmol], powdered molecular sieves 4Å [800mm] and dichloromethane [4mL], followed finally by the addition of methyl trfluoromethanesulphonate [95.74µL, 846µmol]. The reaction vessel was sealed and the reaction mixture agitated at ambient temperature for five hours. The resin was then washed as standard and collected and dried on a sintered funnel. In a reaction vessel resin **56** was then combined with a 5% hydrazine hydrate(55%/H_2O)/DMF [5mL] solution and agitated at ambient temperature for 4h. The DMF solution was filtered from the resin and the resin then further washed with DMF [7mL]. The filtrates were combined and the solvent removed *in vacuo*. The residue was taken up in minimal dichloromethane and passed through a plug of silica (eluent; DCM, TLC: CH_2Cl_2:MeOH, 20:0.3). The combined fractions were concentrated, residue **57** was then taken up in 1,2-dichloroethane [3mL] and reacted with acetylchloride [46µL, 648µmol] in the presence of DMAP [84mg, 684µmol] for three hours at ambient temperature. The reaction was diluted with chloroform [20mL] and washed with saturated citric acid solution [2 x 20mL], saturated sodium hydrogen carbonate solution [2 x 20mL] and saturated brine solution [2 x 20mL]. The organic layer was separated, dried over Na_2SO_4 and concentrated to give a white solid residue. The residue was purified by column chromatography (0.5%

MeOH/DCM, v/v) to give 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[4,6-*O*-benzylidene-2-*O*-pivaloyl-3-*O*-(2,3,4,6-tetra-*O*-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-β-D-glucopyranoside **58** (213mg, 76.3%). R_f = 0.57 (66% ethylacetate/petroleum ethers, v/v), ES-MS *m/z* (ion, intensity) 1486.29 ($[M+H]^+$ 100%)

In a cognate experiment to experiment **58**, compound **47** was substituted with compound **43** (the experiment employing resin **53** (425mg, 0.199mmol/g)), to afford 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[4,6-*O*-benzylidene-2-*O*-(4-chlorobenzoyl)-3-*O*-(2,3,4,6-tetra-*O*-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-β-D-glucopyranoside **59** (96mg, 34%), R_f = 0.23 (1.64% methanol/dichloromethane, v/v), ES-MS *m/z* (ion, intensity) 1543.29 ($[M+H]^+$ 100%)

In a further cognate experiment to experiment **58**, compound **47** was substituted with compound **50** to afford 2-amino-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-β-D-glucopyranoside **60**, R_f = 0.5 (1.96% methanol/dichloromethane, v/v), ES-MS *m/z* (ion, intensity) 1360.73 ($[M+H]^+$ 100%)

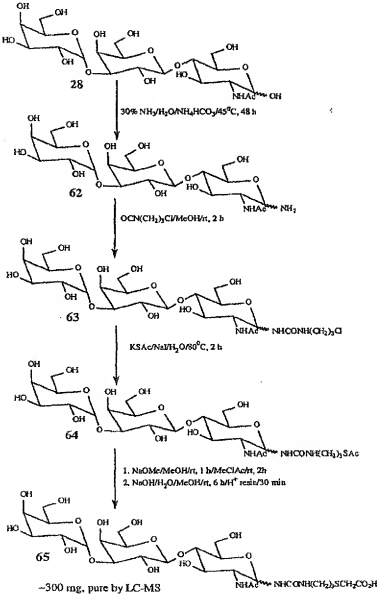
Synthesis of 2-Acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[4,6-O-benzyli-dene-3-O-(2,3,4,6-tetra-O-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyrano-syl)]-β-D-glucopyranoside (**61**)

2-Acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[4,6-*O*-benzyli-dene-2-*O*-pivaloyl-3-*O*-(2,3,4,6-tetra-*O*-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-β-D-glucopyranoside **58** [288mg, 188μmol] was suspended in a solution of NaOMe/MeOH [0.13M, 10mL] to which was added acetonitrile [5mL]. The reaction was heated at 70°C until TLC indicated that the reaction had gone to completion (4-5 days). The reaction mixture was then concentrated and taken up in dichloromethane [20mL] and washed with 10% citric acid solution [2 x 20mL] and saturated brine solution [2 x

20mL]. The organic layer was separated, dried over Na_2SO_2 and the solvent removed *in vacuo* to provide a solid white residue. The residue was purified by preparative thin layer chromatography (eluent: 13% Acetone/DCM) to give 2-Acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[4,6-*O*-benzyli-dene-3-*O*-(2,3,4,6-tetra-*O*-(4-chlorobenzyl)-α-D-galactopyranosyl)-β-D-galactopyranosyl)]-β-D-glucopyranoside **61** [189mg, 69%]. R_f 0.24 (1.47% MeOH/DCM); ES-MS *m/z* (ion, intensity) 1403.29 ($[M+H]^+$, 100%)

Synthesis and Immobilisation of Gal-α-(1-3)-Gal-β-(1-4)-GlcNAc-Linker Conjugate.

Scheme 9: Synthesis of Gal-α-(1-3)-Gal-β-(1-4)-GlucNAc-conjugate



Example 11: Synthesis of Sugar-Linker Conjugate

2-Acetamido-2-deoxy-4-O-[3-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-D-glucopyranosylamine (**62**)

A solution of 2-Acetamido-2-deoxy-4-*O*-[3-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-D-glucopyranose (1 g, 1.8 mmol) **28** and ammonium bicarbonate (0.15 g, 1.9 mmol) in 30% aqueous ammonia (20 mL) was left to stir at 40°C for 48 h. The reaction mixture was then freeze dried to give **62** (1.0 g, ~80% yield by tlc) as a white solid.

Tlc R_f 0.2 (AcN : water, 3 : 1)

1-N-(3-chloropropyl)-1-N'-ureido-2-acetamido-2-deoxy-4-O-[3-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-D-glucopyranoside (**63**)

To a solution of **62** (0.35 g, 6.5 mmol) in methanol (5 mL), was added, 3-chloropropylisocyanate (0.1 g, 0.84 mmol). The reaction mixture was then left to stir at room temperature overnight. The reaction contents was evaporated to dryness and the remaining residue was dissolved in water* (~3 mL) and loaded on to a C-18 Sep-pack column (5 g). The column was eluted** with water (50 mL) followed by 25% methanol in water (50 mL). The methanol fractions were combined and evaporated to dryness giving pure **63** (350 mg, ~80% yield) as a white solid.

Tlc R_f 0.6 (AcN : water, 3 : 1)
M+H found 664
HPLC R_t 4.0 and 4.5 min for α/β anomers (linear gradient: 5% AcN to 20% AcN over 15 min, C-18 column)

1-N-(3-acetoxythiopropyl)-1-N'-ureido-2-acetamido-2-deoxy-4-O-[3-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-D-glucopyranoside (**64**)

A mixture of **63** (0.2 g, 0.30 mmol), sodium iodide (0.1 g, 0.67 mmol) and potassium thioacetate (0.2 g, 1.74 mmol) in water (10 mL) was left to stir at 80°C for 2 h. The reaction mixture was then cooled to room temperature and concentrated to 5 ml. The concentrate was loaded on to a C-18 Sep-pack column (5 g) which was then eluted with water (100 mL) followed by 25% methanol in water (100 mL). The methanol fractions were combined and evaporated to dryness to give pure **64** (0.18g, ~85% yield) as a white solid.

Tlc R_f 0.6 (AcN : water, 3 : 1)
M+H found 703
HPLC R_t 5.5 and 6.0 min for α/β anomers (linear gradient: 5% AcN to 20% AcN over 15 min, C-18 column)

1-N-[3-(methyl carboxymethythio)-propyl]-1-N'-ureido-2-acetamido-2-deoxy-4-O-[3-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-D-glucopyranoside (**65**)

To a solution of sodium methoxide (14 mg, 0.26 mmol) in methanol (3 mL), was added **64** (110 mg, 0.24 mmol). The reaction mixture was stirred at room temperature for 20 min and then methyl bromoacetate (50 mg, 0.30 mmol) was added. The resultant mixture was left to stir at room temperature for 2 h. The reaction mixture was quenched with acetic acid (200 µL) and then evaporated to dryness. The residue was dissolved in water (2 mL) and loaded on to a C-18 Sep-pack column (5 g). The column was eluted with water (50 ml) followed by 50% methanol in water (50 mL). The methanol fractions were combined and evaporated to dryness giving **65** (100.8 mg, 90% yield) as a white solid.

Tlc R_f 0.65 (AcN : water, 3 : 1)
M+H found 734, M+Na found 755

1-N-[3-(carboxymethylthio)-propyl]-1-N'-ureido-2acetamido-2-deoxy-4-O-[3-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-D-glucopyranoside (**66**)

A solution of **65** (300 mg, 0.41 mmol) and potassium hydroxide (30 mg, 0.53 mmol) in 30% aqueous methanol (15 mL) was left to stir at room temperature for 4 h. The reaction mixture was diluted to 50 mL with methanol and then neutralised with IR-120 H^+ resin. The suspension was then filtered and the filtrate evaporated to dryness leaving **66** (295 mg, 100% yield) as a white solid.

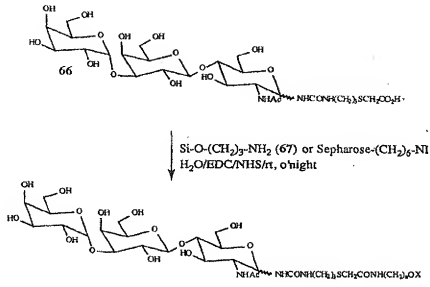
Tlc R_f 0.30 (AcN : water, 3 : 1)
M+H found 719

Notes

*Milli-Q-Water was used at all times

**Flow rate was one drop/sec at all times

Scheme 10: Coupling of Gal-α-(1-3)-Gal-β-(1-4)-GlucNAc-linker conjugate to propylamino-functionalised silica and hexylamino-functionalised Sepharose



Example 12: Immobilisation of Gal-α-(1-3)-Gal-β-(1-4)-GlucNAc-Linker Conjugate

Preparation of 0.3 mmol propylamido-FmocAla-functionalised silica (67)

To a mixture of FMOC-Ala (2.65 g, 8.5 mmol) and HBTU (3.23 g, 8.5 mmol) in dry DMF (20 mL), was added DIPEA (1.1 g, 8.5 mmol). The mixture was shaken for 2 min and then left to stand for 15 min. The mixture was then added to a suspension of propylamino functionalised silica* (17 g) in dry DMF (20mL). The resultant mixture was shaken end over end for 18 h at room temperature. The mixture was filtered and the silica washed with DMF (3 x 100 mL) followed by methanol (3 x 100 mL). The resin was resuspended in a mixture of methanol (100 mL) and acetic anhydride (50 mL) and then shaken for 2 h (negative ninhydrin test after this time). The suspension was

filtered and the silica was then washed with methanol (4 x 100 mL) and dried. The loading of FMOC-Ala was found to be 0.3 mmol per gram** of silica.

*Silica was washed with DIPEA prior to coupling.

**FMOC-Ala loading was quantitated by cleaving (20% piperidine in DMF) a known quantity of FMOC-Ala capped silica and determining the concentration from the UV absorption of the cleavage product at 290 nm against a standard curve.

Coupling of ***66*** *to propylamido-Ala-functionalised silica* **(68)**

FMOC-Ala modified silica from above was cleaved by the standard method (20% piperidine in DMF, rt, 20 min) to give the corresponding free amino (~0.3 mmol loading) functionalised silica. This was then used for the trisaccharide couplings described below.

Loading 1, ~20 mg of ***F*** *per gram of Ala-capped silica:*

To NHS (235 mg, 2.08 mmol), was added a solution of ***66*** (100 mg, 0.139 mmol) and EDC.HCl (2.15 g, 11.2 mmol) in water (10 mL). The resulting solution was added to a suspension of Ala-capped silica (5 g) in water (~10 mL). The suspension was left to shake at room temperature for 3 h, at which time no trisaccharide was present in the filtrate, by tlc. The suspension was then drained, washed with water (4 x 50 ml), dilute sodium bicarbonate solution (3 x 50 ml) and again with water (3 x 50ml). The silica was then resuspended in methanol/acetic anhydride (30 ml, 3:1) and left to shake for 1 h (negative ninhydrin test after this time). The suspension was then drained and the silica washed with methanol (4 x 50 ml) to give the trisaccharide capped silica.

Loading 2, ~5.0 mg of ***66*** *per gram of Ala-capped silica:*

66 (25 mg, 0.034 mmol), NHS (100 mg, 0.884 mmol), EDC.HCl (1.2 g, 6.25 mmol),

and Ala-capped silica (5 g).
Prepared as described for loading 1.

*Loading 3, ~0.5 mg of **66** per gram of Ala-capped silica*:

66 (2.5 mg, 0.0034 mmol), NHS (30 mg, 0.265 mmol), EDC.HCl (130 mg, 0.677 mmol),
and Ala-capped silica (5 g).
Prepared as described for loading 1.

*Coupling of **66** to hexylamino-functionalised Sepharose (EAH Sepharose 4B)* (**69**)

*Loading, ~3.5 to 6.0 mg of **66** per mL of EAH Sepharose*:

EAH Sepharose (5 mL) was washed with water (3 x 50 ml) and then suspended in water (5 ml). To the suspension a solution of **66** (94 mg, 0.131 mmol), EDC.HCl (1.55 g, 8.10 mmol) and NHS (290 mg, 2.57 mmol) in water (15 mL) was added. The reaction mixture was left to shake overnight at room temperature. Tlc of the filtrate showed no **66** present after this time. The reaction contents were drained and the resin was washed with water (3 x 50 mL). The modified Sepharose was then stored as a concentrated suspension in 5% ethanol in water (5 mL).

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

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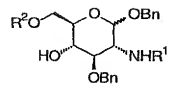
CLAIMS

1. A glucosamine compound of general formula I:

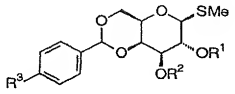


I

in which R^1 is H or acetyl and R^2 is benzyl or 4-chlorobenzoyl,

with the proviso that when R^2 is benzyl, R^1 is not acetyl.

2. A protected monosaccharide building block of general formula II:



II

in which R^3 is methoxy or methyl;

R^1 is H, benzoyl, pivaloyl, , 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methylbenzoyl, benzyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-chlorobenzyl, 4-acetamidobenzyl, or 4-azidobenzyl; and

R^2 is H, Fmoc, benzoyl, pivaloyl, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methylbenzoyl, benzyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-chlorobenzyl, 4-acetamidobenzyl, or 4-azidobenzyl.

3. A protected monosaccharide building block according to claim 2, in which

R^3 is H, R^1 is benzoyl, pivaloyl, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, benzyl, 3,4-methylene-dioxybenzyl, 4-methoxybenzyl, 4-chlorobenzyl, 4-acetamidobenzyl, or 4-azidobenzyl, and

R^2 is Fmoc, benzoyl, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methylbenzoyl, benzyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-chlorobenzyl, 4-acetamidobenzyl, or 4-azidobenzyl,

with the provisos that

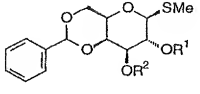
(a) when R^1 is acetyl, R^2 is not chloroacetyl or acetyl, and *vice versa*;

(b) when R^2 is levulinoyl, R^1 is not benzoyl, and *vice versa*; and

(c) when R^1 is benzoyl, R^2 is not benzoyl, and *vice versa*.

4. A protected monosaccharide building block according to claim 2 or claim 3, in which R^2 is Fmoc, and R^1 is benzoyl, pivaloyl, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methylbenzoyl, benzyl, 3,4-methylene-dioxybenzyl, 4-methoxybenzyl, 4-chlorobenzyl, 4-acetamidobenzyl, or 4-azidobenzyl.

5. A protected monosaccharide building block according to any one of claims 2 to 4, in which the compound is of general formula III:



III

in which R^1 is pivaloyl, benzoyl, 4-chlorobenzoyl, 4-methoxybenzyl, or 3,4-methylenedioxybenzyl, and

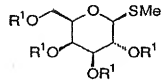
R^2 is H, Fmoc, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methoxybenzyl, or 3,4-methylenedioxybenzyl, with the proviso that if R^1 is benzoyl, R^2 is not levulinoyl.

6. A protected monosaccharide building block according to claim 5, in which the compound is a galactopyranoside, R^1 is 4-chlorobenzoyl, pivaloyl or acetyl, and R^2 is Fmoc or H.

7. A protected monosaccharide building block according to claim 5, in which R^1 is 4-chlorobenzoyl and R^2 is chloroacetyl.

8. A protected monosaccharide building block according to claim 5, in which both R^1 and R^2 are 3,4-methylenedioxybenzyl.

9. A galactopyranoside compound of general formula IV:

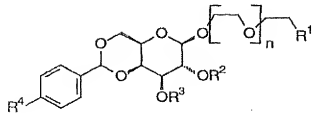


IV

in which each R^1 is independently 4-chlorobenzyl, 4-azidobenzyl, 4-*N*-acetamidobenzyl, 4-methylbenzyl, 3,4-methylenedimethoxybenzyl, or 2-nitrobenzyl.

10. A galactopyranoside according to claim 9, in which each R^1 is 4-chlorobenzyl.

11. A polyethyleneglycol (PEG)-linked monosaccharide of general formula V:



V

in which n is an integer from 1-5;

R^1 is a linking group or a group suitable for the formation of a covalent linkage;

R^2 is acetyl, 4-chlorobenzoyl, levulinoyl, pivaloyl, chloroacetate, benzoyl, 4-methybenzoyl;

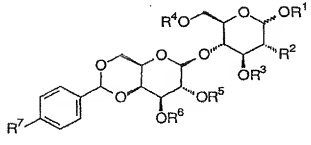
R^3 is H, Fmoc, benzoyl, pivaloyl, 4-chlorobenzoyl, acetyl, chloroacetyl, levulinoyl, 4-methylbenzoyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, or 4-azidobenzyl; and

R^4 is methoxy, H, or methyl.

12. A polyethyleneglycol (PEG)-linked monosaccharide according to claim 11, in which R^1 is selected from the group consisting of halogen, azido, carboxylic acid, thiol, hydroxyl, thioester, xanthate, amido, and dithiocarbamate.

13. A PEG-linked monosaccharide according to claim 11 or claim 12, in which n is 2, R^1 is thiobenzoate or thiobiphenylcarbonyl, R^2 is 4-chlorobenzoyl, R^3 is H, and R^4 is H.

14. A compound of general formula VI:



VI

in which R^7 is H, methoxy or methyl;

R^1 is aryl, substituted aryl, benzyl, substituted benzyl, alkyl, substituted alkyl, PEG, or substituted PEG;

R^2 is acetamido or amino;

R^3 and R^4 are independently benzyl, substituted benzyl, silylether or acyl;

R^5 is 4-chlorobenzoyl, benzoyl, pivaloyl, acetyl, levulinoyl or 4-methylbenzoyl; and

R^6 is a substituted or unsubstituted pyranosyl or furanosyl sugar, H, Fmoc, acetyl, chloroacetyl, levulinoyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, or 4-azidobenzyl.

15. A compound according to claim 14, in which the anomeric configuration of the the glucosamine moiety is α; R^3 is benzyl, R^4 is benzoyl and R^7 is H, R^2 is optionally acetamido, amino, or *N*-phthalimido, R^5 is optionally 4-chlorobenzoyl, benzoyl, pivaloyl, acetyl, levulinoyl or 4-methylbenzoyl, and R^6 is a substituted or unsubstituted pyranosyl or furanosyl sugar, H, Fmoc, acetyl, chloroacetyl, levulinoyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, or 4-azidobenzyl.

16. A compound according to claim 14, in which the anomeric configuration of the the glucosamine moiety is β; R^1 is benzyl and R^7 is H, R^2 is acetamido, amino, or *N*-phthalimido; R^3 and R^4 are independently benzyl, substituted benzyl, silylether or acyl; R^5 is 4-chlorobenzoyl, benzoyl, pivaloyl, acetyl, levulinoyl or 4-methylbenzoyl, and R^6 is a substituted or unsubstituted pyranosyl or furanosyl sugar, H, Fmoc, acetyl, chloroacetyl, levulinoyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, or 4-azidobenzyl.

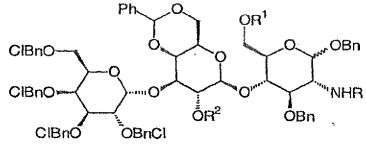
17. A compound according to claim 14, in which the anomeric configuration of the the glucosamine moiety is α; R^1, R^3, and R^4 are benzyl or substituted benzyl, and R^7 is H, R^2 is acetamido, amino, or *N*-phthalimido, R^5 is pivaloyl, 4-chlorobenzoyl, benzoyl, or levulinoyl, and R^6 is a substituted or unsubstituted pyranosyl or furanosyl sugar, H, Fmoc, acetyl, chloroacetyl, levulinoyl, 3,4-methylenedioxybenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, or 4-azidobenzyl, with the proviso that when R^3 and R^4 are benzyl, R^5 is not acetyl or benzoyl.

18. A compound according to claim 14, in which the anomeric configuration of the the glucosamine moiety is β; R^1 is benzyl, R^2 is amino or acetamido, R^3 and R^4 are benzyl, R^5 is 4-chlorobenzoyl, pivaloyl or acetyl, R^6 is Fmoc or H, and R^7 is H.

19. A compound according to claim 14, in which the anomeric configuration of the the glucosamine moiety is α; R^1 is benzyl, R^2 is acetamido, R^3 is benzyl, R^4 is benzoyl

or benzyl, R^5 is 4-chlorobenzoyl, R^6 is H or 4-chloroacetyl and R^7 is H.

20. A compound according to claim 14, in which the compound is a trisaccharide of General Formula VII:



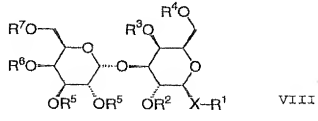
VII

in which R is H or acetyl; R^1 is hydrogen, benzyl, benzoyl or p-chlorobenzoyl; and R^2 is hydrogen, 4-chloro-benzoyl, acetyl, benzoyl or pivaloyl.

21. A compound according to claim 20, in which the anomeric configuration of the reducing end of the trisaccharide is α, R is acetyl, R^1 is benzoyl, 4-chlorobenzoyl or H, and R^2 is 4-chlorobenzoyl or H.

22. A compound according to claim 20, in which the anomeric configuration of the reducing end of the trisaccharide is β, R is acetyl or H, R^1 is benzyl, and R^2 is H, 4-chlorobenzoyl, pivaloyl or acetyl.

23. A compound of general formula VIII:



VIII

in which R^5, R^6 and R^7 are independently H, 4-chlorobenzyl, 4-methoxybenzyl, 4-methylbenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl;

X is O, S, or N;

R^1 is alkyl, substituted alkyl, aryl, substituted aryl, PEG or substituted PEG;

R^2 is levulinoyl, 4-chlorobenzoyl, benzoyl, 4-methylbenzoyl, acetyl or pivaloyl; and

R^3 and R^4 either combine to form a benzylidene ring, which may optionally be substituted at the 4 position by mthyl or methoxy, or R^3 and R^4 are independently H, benzyl or substituted benzyl.

24. A compound according to claim 23, in which R^5 is 4-chlorobenzyl, 4-methoxybenzyl, 4-methylbenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl, and R^6 and R^7 combine to form a benzylidene or substituted benzylidene ring; X is O, S, or N; R^1 is alkyl, substituted alkyl, aryl, substituted aryl, PEG, substituted PEG, acyl or substituted acyl; and R^2 is levulinoyl, 4-chlorobenzoyl, benzoyl, 4-methylbenzoyl, acetyl or pivaloyl.

25. A compound according to claim 23, in which X is oxygen; R^1 is 3,4-methylenedioxybenzyl; R^2 is H, 4-chlorobenzoyl, pivaloyl, acetyl, levulinoyl, benzoyl or chloroacetyl; R^3 and R^4 either combine to become a benzylidene ring or are independently H, benzyl or substituted benzyl; and R^5, R^6 and R^7 may be H, benzyl, 4-chlorobenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl.

26. A compound according to claim 23, in which X is oxygen; R^1 is 2-[2-(2-thiobenzoyl)-ethoxy)ethyl or 2-[2-(2-thiobiphenylcabonyl)ethoxy]; R^2 is H, 4-chlorobenzoyl, pivaloyl, acetyl, levulinoyl, benzoyl or chloroacetyl; R^3 and R^4 combine to form a benzylidene ring, or are independently H, benzyl, 4-chlorobenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl; R^5 is H, benzyl, 4-chlorobenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl; and R^6 and R^7 combine to become a benzylidene ring or are

independently H, benzyl, 4-chlorobenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl.

27. A compound according to claim 23, in which X is sulphur; R^1 is alkyl, substituted alkyl, aryl or substituted aryl; R^3 and R^4 combine to form a benzylidene ring; R^5, R^6 and R^7 are benzyl; and R^2 is levulinoyl, 4-chlorobenzoyl, benzoyl, acetyl or pivaloyl,

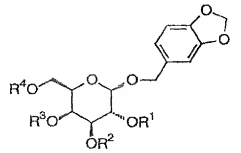
with the proviso that when R^1 is phenyl, R^2 is not levulinoyl.

28. A compound according to claim 23, in which X is oxygen; R^1 is 2-[2-(2-thiobenzoyl)ethoxy)ethyl or 2-[2-(2-thiobiphenylcabonyl)ethoxy]; R^2 is H or 4-chlorobenzoyl; R^3 and R^4 are H or combine to form a benzylidene ring; R^5 is H or 3,4-methylenedioxybenzyl; and R^6 and R^7 are both H, or combine to form a benzylidene ring.

29. A compound according to claim 23, in which X is S, R^1 is methyl; R^2 is 4-chlorobenzoyl; R^3 and R^4 combine to form a benzylidene ring; and R^5, R^6 and R^7 are each 4-chlorobenzyl.

30. A compound according to claim 23, in which X is oxygen; R^1 is 3,4-methylenedioxybenzyl; R^2 is 4-chlorobenzoyl or H; R^3 and R^4 combine to form a benzylidene ring or are both H; and R^5, R^6 and R^7 are independently 4-chlorobenzyl or H.

31. A compound of general formula IX:



IX

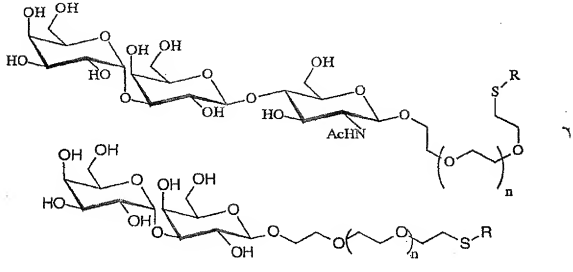
in which R^1 is 4-chlorobenzoyl, pivaloyl, acetyl, levulinoyl, benzoyl or chloroacetyl;

R^2 is H, benzyl, 4-chlorobenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, azidobenzyl, 3,4-methylenedioxybenzyl, Fmoc, levulinoyl, acetyl or chloroacetyl; and

R^3 and R^4 combine to form a benzylidene ring, or are independently H, benzyl, 4-chlorobenzyl, 4-methoxybenzyl, 4-acetamidobenzyl, azidobenzyl or 3,4-methylenedioxybenzyl.

32. A compound according to claim 31, in which R^1 is 4-chlorobenzoyl, R^2 is H, and R^3 and R^4 combine to form a benzylidene ring.

33. A polyethyleneglycol(PEG)-linked disaccharide of General Formula X or a trisaccharide of General Formula XI:

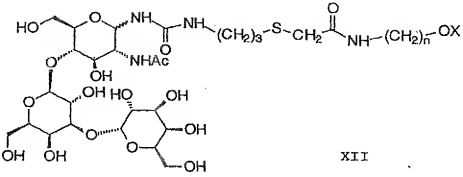


XI

in which R is hydrogen or acyl, and n is an integer of from 1 to 3.

34. A compound of Formula XI according to claim 33, which is 2-[2-(2-thiobiphenylcarboxamidoxy]-ethyl 3-O-(α-D-galactopyranosyl)-α-galactopyranoside.

35. A compound of general formula XII:



XII

in which X is a solid support, and n is an integer of from 3 to 6.

36. A compound according to claim 35, in which X is Sepharose.

37. A compound according to claim 35, in which X is silica gel.

38. A method of synthesis of a disaccharide or trisaccharide, comprising the step of using a compound according to any one of claims 1 to 32 as an intermediate.

39. A method according to claim 38, in which the disaccharide or trisaccharide is selected from the group consisting of

(a) a compound of General Formula X, General Formula XI or General Formula XII;

(b) α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-*N*-acetyl-D-glucosamine (Galα(1→3)Galβ(1→4)GlcNAc);

(c) α-D-galactopyranosyl-(1→3)-β-D-galactopyranose (Galα(1→3)Gal); and

(d) β-D-galactopyranosyl-(1→4)-*N*-acetyl-D-glucosamine (Galβ(1→4)GlcNAc).

40. A method according to claim 38 or claim 39, in which the compound is of General Formula X or XI, and the intermediate compound is of General Formula V.

41. A method according to claim 38, in which the compound is of General Formula VI, and the intermediate compound is of General Formula I.

42. A method of preventing or reducing a hyperacute rejection response associated with xenotransplantation, comprising the step of administering an effective dose of thioalkyl Galα-(1→3)Gal or thioalkyl Galα(1→3)Galβ(1→4)GlcNAc to a subject in need of such treatment.

43. A method of preventing or reducing hyperacute rejection associated with xenotransplantation, comprising the steps of

a) removing plasma from a patient who is to undergo xenotransplantation;

b) exposing the plasma to thioalkyl Galα(1→3)Gal or thioalkyl Galα(1→3)Galβ(1→4)GlcNAc linked to a solid support, and

c) reinfusing the thus-treated plasma into the patient.

44. A method of depleting anti-Galα(1→3)Gal antibodies from a plasma or serum sample, comprising the step of exposing the plasma or serum to thioalkyl Galα(1→3)Gal or thioalkyl Galα(1→3)Galβ(1→4)GlcNAc linked to a solid support.

45. A method of treatment of *C. difficile* infection, comprising the step of administering an effective amount of α-D-galactopyranosyl-(1→3)-β-D-galacto-pyranosyl-(1→4)-*N*-acetyl-D-glucosamine (Galα(1→3)Galβ(1→4)GlcNAc) or of thioalkyl Galα(1→3)Galβ(1→4)GlcNAc to a subject in need of such treatment.

46. A method according to claim 45, in which the Galα(1→3)Galβ(1→4)GlcNAc) or thioalkyl Galα(1→3)Galβ(1→4)GlcNAc, is linked to a solid support.

47. A method according to claim 45, in which the solid support is a multidentate ligand or a dendrimer compound.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00028

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07H 15/18, 17/04, 15/26, 15/08, 23/00, 15/12, 1/00, 5/04, 3/04; C07D 493/04; A61K 31/702, 31/7016

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Chemical Abstracts, MedLine: substructure for formulae II, , III, V, VI, VII, molecular formulae, difficile, gal, saccharide and similar terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Organic Letters, 2000, Vol. 2, No. 17, pp 2713-2715; S. Sugiyama et al.: "A Convenient Preparation of Glycosyl Chlorides from Aryl/Alkyl Thioglycosides" See particularly compound 30, p 2714	11
X	PubMed (Medline) Abstract ID 8690209: Gastroenterology 1996 Aug;111(2):433-8; Castagliuolo I et al.: "A receptor decoy inhibits the enterotoxic effects of Clostridium difficile toxin A in rat ileum." See Abstract	45-47
X	PubMed (Medline) Abstract ID 9258442: Bioconjug Chem 1997 Jul-Aug;8(4):466-71; Nilsson UJ et al.: "Immobilization of reducing sugars as toxin binding agents." See Abstract	45-47

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"B" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 March 2001	26 MAR 2001
Name and mailing address of the ISA/AU	Authorised officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	G. D. HEARDER Telephone No : (02) 6283 2553

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00028

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PubMed (Medline) Abstract ID 8922955: Glycobiology 1996 Sep;6(6):599-609; Teneberg S et al.: "Molecular mimicry in the recognition of glycosphingolipids by Gal alpha 3 Gal beta 4 GlcNAc beta-binding Clostridium difficile toxin A, ..." See Abstract	45
X	PubMed (Medline) Abstract ID 8964394: Gastroenterology 1996 Jun;110(6):1704-12; Pothoulakis C et al.: "A human antibody binds to alpha-galactose receptors and mimics the effects of Clostridium difficile toxin A in rat colon." See Abstract	45
X	PubMed (Medline) Abstract ID 1670930: Infect Immun 1991 Jan;59(1):73-8; Tucker KD et al.: "Toxin A of Clostridium difficile binds to the human carbohydrate antigens I, X, and Y." See Abstract	45
X	PubMed (Medline) Abstract ID 3115180: Arch Biochem Biophys 1987 Aug 15;257(1):217-29; Clark GF et al.: "Toxin A from Clostridium difficile binds to rabbit erythrocyte glycolipids with terminal Gal alpha 1-3Gal beta 1-4GlcNAc sequences." See Abstract	45
X	PubMed (Medline) Abstract ID 3112015: Infect Immun 1987 Aug;55(8):1873-7; Krivan HC et al.: "Purification of Clostridium difficile toxin A by affinity chromatography on immobilized thyroglobulin." See Abstract	45
X	PubMed (Medline) Abstract ID 3744552: Infect Immun 1986 Sep;53(3):573-81; Krivan HC et al.: "Cell surface binding site for Clostridium difficile enterotoxin: evidence for a glycoconjugate containing the sequence Gal alpha 1-3Gal beta 1-4GlcNAc." See Abstract	45
A	Transplantation, Vol. 57, 959-963, No. 6, March 1994; Francisca A et al.: "Protection of pig kidney (pk15) cells from the cytotoxic effect of anti-pig antibodies by galactosyl oligosaccharides." See Table 1, # 2 and 17; Table 2, #2 and 16; Table 3, # 1 and 3; and discussion	42-44
A	Transplantation, Vol. 65, 172-179, No. 2, 27 January 1998; Y Xu et al.: "Removal of anti-porcine natural antibodies form human and nonhuman primate plasma in vitro and in vivo by ... ." See p 172 (footnote), and p 178-9	42-44
A	J. Am. Chem. Soc. 31 August 1999, Vol. 121, No. 36, 8174-8181; J-Q Wang et al: "Enhanced inhibition of human anti-gal antibody binding to mammalian cells by synthetic -gal epitope polymers." See p 9177-8	42-44
A	WO 93/03735 A1 (ALBERTA RESEARCH COUNCIL) 4 March 1993 See fig 1A #1 and 4, claims	42-44
A	WO 99/52561 A1 (BAXTER INTERNATIONAL INC.) 21 October 1999 See Fig 14, 21 and claims	42-44

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00028

Box I **Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :

because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos : 9, 23, 31, 33

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

For economic reasons the search was necessarily restricted, see supplemental sheet.

3. ☐ Claims Nos :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00028

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: I. 2.

As the scope of the many formulae was quite broad, the search was necessarily restricted for economic reasons. The search for compounds of Formulae IV, VIII, IX, X, X1, was restricted to the exemplification disclosed in the specification.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU01/00028

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9303735	AU	25059/92	EP	661980	IL	120453
		IL	102916	US	5651968	US	5695759
		US	5767093	US	5977079		
WO	9952561	AU	35645/99				

END OF ANNEX